A. H. O'Nese.

Bryn Mawr Hospital.

THE PARASITIC AMŒBÆ OF MAN

BY

CHARLES F. CRAIG, M.D.

CAPTAIN, MEDICAL CORPS, UNITED STATES ARMY

FROM THE BACTERIOLOGICAL LABORATORY OF THE ARMY MEDICAL SCHOOL, WASHINGTON, D. C., AND THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH NEW YORK CITY

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PREFACE.

THE great importance of amœbic infections of the intestine and liver in man, and the frequency with which such infections occur in our tropical possessions, render a work upon the parasitic amœbæ of man of interest to the medical profession of the United States, especially as recent investigations have proven that amœbic dysentery is by no means rare, even in the temperate regions of our own country. Much of the literature treating of this subject is in German, French, and Italian, and but little of it has been translated into English, while nearly all of the recent work done upon the differentiation of species of amœbæ has been accomplished by German investigators, and published only in German periodicals and books. No monograph has appeared in English giving in detail the work accomplished by numerous students of the amœbæ which are parasitic in man, and for this reason I have thought that such a work would prove of value to the profession of this country, especially to medical officers of the Army, Navy, and Marine Hospital Service, and to public health officers, as well as to physicians practicing in the infected portions of the United States.

During my service in the United States military hospitals, notably the U. S. Army General Hospital, Presidio of San Francisco, Cal., and the U. S. Army Division Hospital, Manila, P. I., while a member of the U. S. Army Board for the Study of Tropical Diseases, material for the study of amœbic dysentery was nearly always available, and could be utilized to the best advantage. Because of most favorable opportunities I have for nearly twelve years devoted much study to the amœbæ occurring in this form of dysentery and much of the data given in this monograph is based upon the personal observations of hundreds of cases of amœbic dysentery, both clinically and upon the autopsy table, and upon the study of thousands of preparations containing these organisms.

Until the work of Schaudinn, published in 1903, no clear distinction had been made between the various species of amœbæ infesting man, but he was able to differentiate two species, one causing a form of dysentery, to which he gave the name, *Entamœba histolytica;* and another, a harmless commensal of man, which he named *Entamœba coli.* I was able to confirm his work while studying amœbæ at the U. S. Army General Hospital in San Francisco, and my observations were published in 1905, being the first published which confirmed Schaudinn's work, so far as I know. Regarding my studies upon *Entamœba histolytica,* Hartmann, in the Archives für Protis-

tenkunde, Vol. XVIII, No. 2, 1909, p. 207, says: "Since the publication of Schaudinn, *Entamœba histolytica* has been accurately described only by Craig (1908), Werner (1908), and myself (1906–1908), and Schaudinn's description thereby confirmed." As a matter of fact, I had confirmed Schaudinn's description and published my results in 1905, and the paper referred to by Hartmann was only a continuation of that paper. At the same time I was able to follow the life cycle of both *Entamœba histolytica* and *Entamœba coli* and to demonstrate the truth of Schaudinn's description of the methods of reproduction of *Entamœba histolytica.* Regarding my illustrations of the latter process, Hartmann (*ibid.*) says: "On the other hand, he alone, until now, has published illustrations of chromidial and cyst formation which are capable of recognition," thus confirming the accuracy of these observations.

More recently (1910–1911) I have been able to confirm the observations of Viereck and Hartmann upon *Entamœba tetragena* as regards its morphology and life cycle, and have demonstrated the presence of this species in patients suffering from dysentery contracted in the Philippine Islands and in the United States.

In the following pages I have endeavored to give a detailed description of the various species of amœbæ which have been described as parasitic in man,

especially as regards morphology, life cycle, methods of differentiation, and relation to disease, and to include everything of value resulting from the investigation of these interesting and important parasites. I have tried to be conservative in the conclusions drawn from my own work and that of others, for there are many questions still unsettled regarding the status of some of the species of amœbæ described, and it is more than probable that further research will result in the elimination of several species which are to-day held by some observers to be valid.

I desire to here express my deepest gratitude to Brigadier General George H. Torney, Surgeon General of the United States Army, for constant encouragement and assistance, and for opportunities afforded me for research work, and to Dr. Simon Flexner, Director of the Rockefeller Institute for Medical Research, for many favors extended while working in that Institute and for his interest in the publication of this work. My thanks are also due Major Frederick F. Russell, U. S. Army, Director of the Bacteriological Laboratories of the Army Medical School, and to those authors whose illustrations are here reproduced, and from whose investigations I have profited. Credit has been given in every instance, I believe, but if not, pardon is asked for an unintentional omission.

CHARLES F. CRAIG.

WASHINGTON, D. C., July 10, 1911.

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HISTORICAL.

THE history of the development of our knowledge of the parasitic amœbæ of man is of interest as showing the length of time required in the study of this class of parasites before very definite results were obtained, and even to-day our knowledge is far from complete in regard to some of the species described and their relation to disease.

Probably the first investigator to observe amœbæ in the feces of man was Lambl, of Prague, who in 1860 described organisms occurring in the feces of a child which he interpreted as amœbæ, but he attached no great importance to their presence, although the child was suffering from severe diarrhœa at the time. However, a careful perusal of this paper shows that he had his suspicions that the organisms were the cause of the diarrhœa, so that to him belongs the credit of not only first describing the morphology of these parasites, but

also of demonstrating their presence in association with diarrhœa. Following Lambl in 1870, Lewis and Cunningham found amœbæ in the feces of nearly 20 per cent. of cholera patients studied in India. These amœbæ were described by them as large, granular, vacuolated, amœboid organisms, which multiplied by gemmation. They also found them in patients who were suffering from other diseases and in healthy individuals. These investigators did not consider the parasites of any pathological significance, but interpreted them as stages in the development of flagellates.

It was not until 1875 that a really accurate description was given of an amœba occurring in man. At that time Loesch, of St. Petersburg, had the good fortune to observe a patient suffering from dysentery in whose feces numerous amœboid organisms were constantly found. The history of this case shows it to have been one of typical amœbic dysentery, several relapses occurring at intervals, during which the amœbæ were demonstrated in the feces. The description of the organisms, as given by Loesch, makes it evident that they were pathogenic in nature and it is probable that he actually studied *Entamœba histolytica,* the pathogenic species afterward differentiated by Schaudinn. To this parasite he gave the name "*Amœba coli.*" He was successful in

producing ulceration of the intestine in a dog which he injected with feces containing these organisms, so that to this author belongs the credit of first experimentally producing dysentery in the lower animals by the injection of material containing a parasitic amœba of man. His researches were followed by further work by Cunningham and by Koch. The former author found amœbæ in the feces of both healthy and diseased individuals and described bodies which he considered to be spores, but he did not attach any pathological importance to the amœbæ. Koch autopsied five cases of dysentery in Egypt, two of them complicated by abscess of the liver. In the ulcers occurring in the intestine he found numerous amœbæ and in sections they were found at the base of the ulcerations. The parasites were also found in the capillaries of the liver, close to the abscess walls. Koch considered that on account of their location they bore some etiological relation to the disease.

Grassi, in 1879, confirmed the presence of amœbæ in human dejecta, but considered them harmless commensals as he found them in both health and disease. His paper was followed by those of Sonsino, Normand, Perroncito, Callandrucio, and Blanchard, all confirming the presence of amœbæ in the stools of patients suffering from diarrhœa or dysentery.

In 1886 Kartulis commenced the publication of a series of articles upon his investigations on amœbic dysentery as observed in Egypt. These contributions will always rank as among the most important which have been published regarding this subject. In his first paper he described amœbæ which he found in all of one hundred and fifty cases of amœbic dysentery, and in later publications he gave his results after the study of five hundred cases. From his extensive experience he concludes that the organisms are the cause of a form of dysentery, often associated with liver abscess, and his thorough and scientific researches may be said to have finally established the etiological relationship of amœbæ to certain forms of dysentery.

Kartulis made some attempts at cultivation and claimed to have been successful in a few instances. His work was well controlled by the examination of sections prepared from the intestine of individuals dying from tuberculosis, typhoid fever, typhus fever, and other diseases. While he found in sections made from the intestine of dysenteric patients that the amœbæ were always present, he never found them in sections from patients dying from other diseases. Despite their great value the researches of this author attracted but little attention in Europe, America, or in the tropics.

Shortly after the publication of the first paper of Kartulis an investigation of dysentery at Prague, by Hlava, resulted in the finding of amœbæ in sixty cases of the disease, his description of the organisms agreeing with that of Kartulis. He experimented upon dogs and cats by injecting feces containing amœbæ into the rectum, obtaining positive results in two dogs out of seventeen and in four cats out of six experimented upon. This paper was followed by those of Massiutin and Pfeiffer, who both found amœbæ in the feces in cases of dysentery.

In America the first investigator to observe amœbæ in a dysentery case was Osler who, in 1890, in a patient suffering from chronic dysentery complicated by liver abscess found amœbæ in the stools which answered in their morphology to those described by Kartulis. His observation was followed in the same year by those of Musser and Stengel, and in 1891, by those of Dock, who confirmed the presence of these organisms in dysenteric stools.

In 1891, Councilman and Lafleur published their classical monograph upon amœbic dysentery, in which they concluded that the disease is a clinical entity and is characterized by definite pathological lesions due to the amœbæ. Their study was based upon fourteen cases of amœbic dysentery, and besides giving a most excellent description of the parasite now known as

Entamœba histolytica, they compared the clinical differences between amœbic dysentery and catarrhal dysentery, as well as the differences in the pathological lesions of the two diseases. They proposed the name *Amœba dysenteriæ* for the organism associated with the lesions which they described, and clearly recognized that other, and perhaps non-pathogenic amœbæ, might infest the intestine of man. It is not too much to say that to these authors we owe a large part of the interest which has been shown in the study of dysentery during recent years.

From 1891 to 1893 confirmatory studies appeared by Cahen, Lutz, Kovacs, and Quincke and Roos. The latter authors, whose work was done at Kiel, concluded that there were at least two varieties of pathogenic amœbæ as well as one variety which was non-pathogenic. In view of the recent separation of two species of pathogenic amœbæ, *Entamœba histolytica* and *Entamœba tetragena,* these observations of Quincke and Roos are of interest, as it is possible that these authors were the first to differentiate these two species.

In 1894, Kruse and Pasquale, who studied amœbic dysentery in Alexandria, Egypt, confirmed the work of Councilman and Lafleur and considered that there existed two species of amœbæ, one pathogenic and the other a harmless inhabitant of the intestinal tract.

They were able to produce typical amœbic dysentery in cats by the injection into the rectum of pus containing amœbæ obtained from a liver abscess.

In 1895, Celli and Fiocca commenced the publication of a series of papers dealing with the amœbæ occurring in man. They did not consider these organisms of etiological significance in dysentery, and their results only added to the confusion which had arisen regarding the existence of pathogenic and non-pathogenic species.

In 1897, Casagrandi and Barbagallo concluded that none of the amœbæ found in the intestine of man were pathogenic, but they separated several species which will be considered in the chapter devoted to classification and nomenclature.

The later investigations of Harris, in the United States, of Strong and Musgrave, and of Musgrave and Clegg in the Philippines, were confirmatory of the etiological importance of amœbæ in the production of dysentery, especially that form prevalent in the tropics and accompanied by abscess of the liver. These investigators were all successful in producing dysentery in animals by material containing the amœbæ occurring in man. In 1902, Jürgens published a monograph upon amœbic dysentery in which he differentiated a pathogenic from a non-pathogenic species, calling attention to the difference in mor-

phology of the two parasites and the occurrence of the pathogenic species only in cases of dysentery.

Although up to this time several investigators had endeavored to separate the parasitic amœbæ of man into species, either pathogenic or non-pathogenic, it must be admitted that until Schaudinn's publication appeared in 1903 no clear description had been given of specific differences in this class of organisms as it is represented in man. The epoch-making paper of Schaudinn gave the results of his work at Rovigno where he pursued a long series of researches upon these organisms. He clearly showed that there occurred in man at least two species of amœba differing in their morphology, methods of reproduction, and life cycle; one, a harmless commensal, occurring both in health and in disease, the other, the cause of a form of dysentery. He was able to follow the entire life cycle of both these organisms and by experiments upon himself showed that one was harmless and that the other when swallowed during a certain stage of development was capable of producing dysentery. He suffered from two attacks of the disease acquired in this manner and four years later died as the result of an abscess of the sigmoid flexure, in all probability produced by the pathogenic amœba with which he had infected himself. To the harmless amœba he gave the name "*Entamœba coli,*" while

to the one producing dysentery he gave the name "*Entamœba histolytica.*" To this investigator undoubtedly belongs the credit of the establishment, upon scientific grounds, of two species of amœba infesting the intestine of man.

In 1905, while serving at the U. S. Army General Hospital in San Francisco, California, where there was always abundant material for the study of amœbic dysentery, I was able to partially confirm Schaudinn's results and later was able to entirely confirm and add to them.

For a long time Schaudinn's work was received with doubt, but at the present time almost every authority, who has had any experience in the study of these organisms, has accepted Schaudinn's classification, and in the recent work of such zoölogists as Lühe, Braun, Hartmann, Minchin, Stiles, Wenyon, Doflein, and Calkins, this classification has received complete recognition, while confirmatory papers have been published by Hartmann, Werner, Viereck, Jürgens, Kartulis, Simon, Wenyon, Fantham, and many others.

The work of Schaudinn had the effect of stimulating anew the study of the amœbæ parasitic in man and since his paper appeared several new species have been described. It is probable that only a few of these are entitled to specific rank, but they will

all be considered in this contribution, the data concerning each being given so far as it is on record. The most important new species was described by Viereck in 1906, who found an amœba, differing from any heretofore described, in cases of dysentery, to which he gave the name *Entamœba tetragena.* His observations have been confirmed by Hartmann, Prowazek and myself, and there can be no doubt but that this is a distinct species of pathogenic amœba. In the same year I described another species of amœba possessing both a flagellate and an amœboid cycle of development to which the name "*Paramœba hominis*" was given. In 1904 Prowazek described a species occurring in the mouth to which he gave the name *Entamœba buccalis.*

In addition to the species just mentioned several others have been described, *i.e., Entamœba tropicalis,* studied by Lesage in 1908; *Entamœba minuta,* described by Elmassian in 1909; and *Entamœba nipponica,* described by Koidzumi in the same year. In addition to these there are a few so-called species which possess little interest or scientific value by reason of imperfect observation or description.

From this summary of the history of the development of our knowledge regarding the parasitic amœbæ of man it will be noted that it has been of slow growth, over fifty years having elapsed since

the observation of Lambl of an organism of this nature in the intestinal discharges of a case suffering from diarrhœal disease. In fact, it may be said that almost all of our exact knowledge concerning the specific differentiation of these parasites dates from the paper of Schaudinn published in 1903, and that greater advance has been made during the past seven years than in the forty-three years preceding. At the present time it is definitely established that several species of amœba are parasitic in man, that some of them are capable of producing typical pathological lesions, and that it is possible to produce similar lesions in some of the lower animals by the feeding or injection of material containing these parasites. In addition the entire life cycle of several of the species has been worked out and the differentiation of these species is based upon the life cycle as well as upon definite morphological characteristics.

In the succeeding pages, under the appropriate headings, further historical details will be given concerning each species.

II.

GENERAL MORPHOLOGY AND BIOLOGY OF AMŒBÆ.

Before considering in detail the morphology and life history of the parasitic amœbæ of man it is necessary to review briefly the general morphology and biology of amœbæ, as ignorance of the structure and life history of saprophytic organisms belonging to this sub-class of the Rhizopoda has resulted in great confusion in the classification of the amœbæ of man. It should be constantly borne in mind that the species occurring in man comprise but a minute number when compared with the multitude of species which are free-living and which are harmless and incapable of a parasitic existence.

The amœbæ belong to the Protozoa, subphylum Sarcodina, class Rhizopoda, sub-class Amœbina, and order Gymnamœbida. These organisms are unicellular in type, all of their functions being performed by the single cell. They occupy the lowest position in the animal kingdom, but in one genus, *Paramœba,* a gradation may be traced to the next higher subphylum, the Mastigophora. They are found very widely distributed as free-living forms and as parasites within man and many of the lower animals.

Collections of stagnant water in almost every region abound in amœbæ, and in the tropics almost every source of water supply is contaminated with these organisms. They have also been found upon green vegetables, especially salad vegetables, and it is not at all improbable that they occur on all garden produce in regions where human excrement is used for fertilizing purposes. Fortunately only a small number of species are pathogenic for man, as otherwise almost every inhabitant of warm regions would suffer from amœbic dysentery.

GENERAL MORPHOLOGY.

Roughly stated these organisms may be said to consist of a mass of protoplasm containing a nucleus and one or more vacuoles, which may or may not be contractile in character. Reproduction occurs by *simple division, schizogony, gemmation,* and *reproduction within a cyst.* They vary in size in different stages of development, measuring from 5 μ to 70 μ or more in diameter, and are spherical or oval in shape when not in motion. The size of the organism alone cannot be used as a method of species differentiation as it varies greatly at different periods of growth. All amœbæ present for description a *cytoplasm* and a *nucleus,* together with certain other

primitive organs which will be mentioned in the general description.

The Cytoplasm.—The body of an amœba is composed of a mass of cytoplasm varying in appearance and structure in different species. As a rule, two distinct portions of this substance may be recognized, an outer portion, known as the *ectoplasm* and which comprises the smaller portion of the cytoplasm, and an inner portion, known as the *endoplasm*. It is in the latter portion that the nucleus lies imbedded.

The *ectoplasm* may be well or illy defined from the endoplasm, and in some species is seen to be composed of minute granules, while in others it appears structureless. In some, the ectoplasm is grayish in color and veil-like in appearance, while in other species it is glass-like in appearance and dense in consistence. Thus in *Entamœba coli* the ectoplasm is difficult to distinguish from the endoplasm and is of very delicate consistence, while in *Entamœba histolytica* the ectoplasm is very distinct, glass-like in appearance, and dense in structure. In some species the ectoplasm can only be differentiated when the organism is in motion, while in others it is impossible to differentiate it at any time. In a few instances the ectoplasm contains coarse granules, the nature of which is unknown.

The Endoplasm.—In all species of amœbæ, if a

high power be used in the examination, the endoplasm is observed to be composed of a delicate meshwork containing within it numerous granules which vary in size in different species. These granules have been termed " microsomes " by some authorities. As a matter of fact they differ in nature, some being due to undigested particles of food, some to chromidia, some to waste materials, while others consist of minute crystals or oil droplets. According to Butschl the entire cytoplasm of these organisms is composed of a fluid substance contained within a meshwork composed of another fluid of different composition.

The endoplasm may be more or less refractive and may contain, besides the nucleus, one or more vacuoles; threads, granules, and masses of chromatin (chromidia); various crystals derived from the surrounding media; bacteria of various kinds; and red blood corpuscles in the case of the pathogenic amœbæ of man.

The NUCLEUS varies in size, structure and shape in different species of amœbæ, and is of considerable service in distinguishing between such species. A visible nuclear membrane is observed surrounding the nucleus in some species, while in others such a membrane cannot be distinguished. In some species a distinct karyosome can be seen, generally situated near the centre of the nucleus, and this sometimes con-

tains a minute *centriole* or *centrosome,* and in one or two species is of service in differentiation. Within the nucleus there occurs a varying amount of *chromatin,* the arrangement of which is of value in distinguishing species. In some species the chromatin is collected in masses upon the nuclear membrane, while in others it is distributed throughout the nucleus in the form of a network enclosing a well defined karyosome. The amount of chromatin varies in different species, in some being almost absent, while in others it constitutes the greater portion of the nucleus. A certain amount of *achromatic substance* which is unstainable is contained within the nucleus. In living specimens the chromatin appears as brightly refractile granules or masses situated within the nucleus or arranged around the nuclear membrane, while in stained specimens it takes the ruby red color characteristic of this substance if any modification of the Romanowsky stain is used.

Besides the nuclear chromatin certain species of amœbæ, when stained, present a large amount of this substance lying within the endoplasm. In such instances the chromatin is distributed throughout the endoplasm in the form of granules or threads or as irregular masses which may be situated near the periphery of the organism. The chromatin situated within the endoplasm is known as *chromidia* or *idio-*

chromidia. The amount of chromatin present within the endoplasm is of considerable value in the differentiation of species.

Besides the *nucleus,* the endoplasm may contain one or more vacuoles which may be contractile or non-contractile in character. The nature of the vacuoles is of great value in distinguishing the parasitic amœbæ found in man from the free-living forms, as a contractile vacuole is not present in the parasitic amœbæ, but is always present in most of the common free-living species. Thus in the genus *Entamœba* a contractile vacuole has never been observed in specimens obtained from the human intestine and examined in the living condition, while in *Amœba limax,* and *Amœba proteus,* common free-living species, a contractile vacuole is always present. It is significant that the amœbæ which have been cultivated from the human intestine show in the cultural forms a contractile vacuole, so far as I have observed. This fact renders it more than probable that these amœbæ were really free-living forms which had been swallowed with food or drink and had passed through the intestinal canal in an encysted condition.

The number and size of the vacuoles varies in different species. Thus in *Entamœba coli* there is seldom more than one or two small vacuoles present, while in *Entamœba histolytica,* a number of vacuoles

may be present, of large size. In the parasitic species found in man, vacuoles are generally present and are digestive in character, but they are never observed to pulsate. Small masses of granular material are often seen within the vacuoles and sometimes stain with neutral red, thus showing that the vacuoles are digestive in character. As a rule the vacuoles are situated near the periphery of the amœbæ, especially when they are contractile, but they change their position with the movements of the organism. In *Amœba proteus*, a free-living form, it is easy to observe the formation and contraction of the vacuole. At first it is situated near the nucleus and is very small, but as nutritive material collects within it, the vacuole grows larger, at the same time moving toward the periphery of the organism. When it arrives at the periphery it suddenly contracts and its contents are rejected from the body of the amœba. Such phenomena are never observed in the parasitic amœbæ of man.

In addition to the vacuoles there are present in the endoplasm of some species of amœbæ, especially those parasitic in man, numerous minute, oval or round bodies, which resemble the spores of malarial plasmodia, in unstained preparations, and which have been described by different authors as *spores*. At a certain stage of the development of *Entamœba coli*, several such bodies may be observed in both living and

stained specimens and represent the nuclei of the daughter amœbæ which are forming within the parent organism. In *Entamœba histolytica* these bodies are often numerous and represent the collections of idiochromidia which form the nuclei of the young parasites which are produced by gemmation.

Besides the vacuoles and the spore-like bodies just noted, there may occur, in the endoplasm of amœbæ, minute *crystals* of various kinds which have been taken up from the surrounding media, as well as bacteria and foreign particles of varying nature. In the parasitic amœbæ found in man the endoplasm often contains red blood cells if intestinal inflammation be present.

GENERAL BIOLOGY.

Amœbæ obtain nutriment by means of pseudopodia which are protruded from the body and surround food materials which are then taken into the body of the organism where they undergo digestion. In these organisms we meet with the most primitive form of digestion, any portion of the cytoplasm being capable of absorbing and digesting food materials. In those amœbæ which possess a contractile vacuole the food material is enclosed within it and digested, the residue being expelled when the vacuole contracts. In those organisms in which a contractile vacuole is absent

there occur numerous digestive vacuoles which perform the same function. In many species of amœbæ, especially those belonging to the genus *Entamœba,* the exact method by which undigested food is gotten rid of is still unknown, but it is probable that such material is simply extruded from the periphery of the organism.

RESPONSE TO STIMULATION.—Amœbæ are capable of responding to stimulation of either *mechanical, chemical,* or *electrical* nature.

Mechanical stimulation is best illustrated by the production of pseudopodia when the organism touches surfaces of any kind, and the withdrawing of the pseudopodia when it is touched with a needle or when it comes in contact with obstacles to locomotion.

Response to *chemical* irritation is noted when a solution containing some substance poisonous to the organism is allowed to come in contact with it. In such instances the pseudopodia are quickly withdrawn and the amœba assumes a spherical shape.

Response to *electrical* stimulation is noted when galvanic currents are passed through water containing amœbæ. The organisms which are motile quickly cease motion, the pseudopodia are withdrawn and a spherical form is assumed. If the currents are of a mild character the organisms renew their movements in a variable time, but if too strong, death ensues.

If an amœba in which several pseudopodia are present is gently touched with a needle upon only one of the pseudopodia, they are all quickly withdrawn, thus proving that the protoplasm of the organism possesses *conductivity.*

MOTILITY.—Aside from reproduction, the most striking phenomenon observed in amœbæ is the power of locomotion. This is rendered possible by a form of motility which, because it is best illustrated in amœbæ, is known as *amœboid motion.* This, the most primitive form of locomotion, consists in the throwing out of *pseudopodia* at the periphery of the organism and the flowing into them of the cytoplasm. Jennings compares it to rolling, the upper surface of the cytoplasm passing forward and turning under at the anterior end of the pseudopodia, thus producing the appearance of the cytoplasm flowing in the direction of motion. By this means an amœba is able to move in any direction, the rate of rapidity of motion varying with the surroundings and the species of organism. Locomotion in any one direction only lasts for a short period of time, and it is not unusual to observe an amœba move in the opposite direction without any apparent reason. When an obstacle is encountered by a pseudopodium it is quickly withdrawn and another is protruded, the organism moving away in another direction.

The protrusion of pseudopodia is not always followed by locomotion for organisms are frequently observed from which pseudopodia are protruded constantly and as constantly withdrawn, but there occurs no change in position. The pathogenic amœbæ are generally more actively motile than the non-pathogenic species.

The pseudopodia are always formed by the ectoplasm and are well defined in some species, while in others no differentiation can be made between the pseudopodium and the endoplasm. This difference in the appearance of the pseudopodia is of some value in distinguishing species.

Pseudopodia vary greatly in size and shape in different species, in some being spinose and very delicate in appearance; in others finger-like and dense in structure; while in still others they are broad and blunt. In some amœbæ they are so characteristic in appearance as to be of great service in species differentiation. Thus in *Entamœba coli* the pseudopodia are blunt and of a delicate veil-like appearance, while in *Entamœba histolytica* they are much longer and finger-like in shape and present a dense, glassy appearance. In some of the free-living forms the pseudopodia are always spinose in shape, differing entirely in appearance from those of the parasitic amœbæ. I have never observed spinose pseudopodia in any of the parasitic amœbæ of man.

The exact mechanism of the origin of the pseudopodia has been studied by many observers, and numerous theories have been brought forward to explain this interesting phenomenon. However, it may be stated that we are yet ignorant of the real mechanism of pseudopodia formation.

In one genus of amœbæ, *i.e., Paramœba,* delicate *flagella* have been observed during certain stages of development. Organisms belonging to this genus possess both an amœboid and flagellate cycle of development. During the amœboid cycle they move about by means of pseudopodia, while during the flagellate cycle locomotion is accomplished by means of a flagellum which apparently takes its origin from some portion of the periphery of the organism.

METHODS OF REPRODUCTION.—Although these organisms, from a morphological standpoint, illustrate the most simple form of animal life, they possess several methods of reproduction, some of them of great complexity. These methods are: *simple division, schizogony, gemmation,* and *reproduction within a cyst.* The first three methods occur during the vegetative stage of existence, the latter, only when conditions arise which are unfavorable to such existence. Schizogony and reproduction within a cyst are confined to certain species, and gemmation appears to be peculiar to *Entamœba histolytica,* but simple division occurs in all species.

Simple Division.—This method of reproduction is asexual in nature and consists of a primary division of the nucleus, which may be either mitotic or amitotic in character, followed by the division of the cytoplasm, two amœbæ being thus produced. This method of reproduction occurs in all species of amœbæ, so far as is known.

Schizogony.—This method of reproduction is also asexual in nature and is accomplished by the division of the karyosome by primitive mitosis, after which the nuclear chromatin becomes collected around the nuclear membrane while the cytoplasm rids itself of all foreign material. When this is completed, the chromatin divides into from four to eight little masses, the nuclear membrane ruptures and the chromatin masses become free in the endoplasm. Finally, the cytoplasm divides into as many parts as there are chromatin masses, and a number of daughter amœbæ are thus formed. This process will be described in detail in the consideration of *Entamœba coli,* and *Entamœba tetragena.*

Gemmation.—This process of reproduction, as observed in amœbæ, was first described by Schaudinn as occurring in *Entamœba histolytica.* In consists in amitotic division of the nucleus, the chromatin being dispersed in the cytoplasm, and collecting at the periphery of the organism where it is budded off,

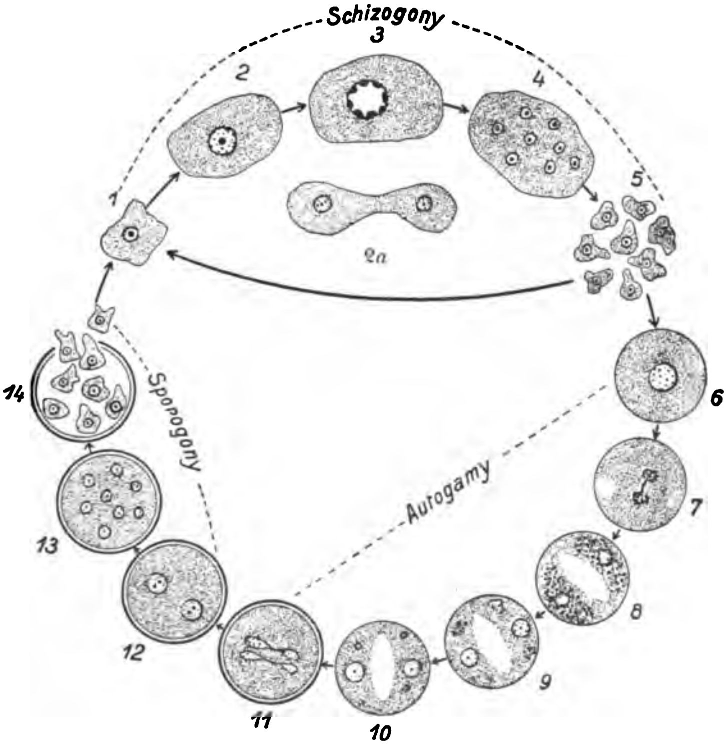

FIG. I.—Diagram illustrating the life-cycle of *Entamœba coli*. (After Hartmann.) 1, young amœba; 2, adult amœba; 2*a*, reproduction by simple division; 3, the division of the chromatin of the nucleus into eight groups collected upon the surface of the nuclear membrane; 4, complete nuclear division; 5, schizogony, or division of the amœba into eight daughter amœbæ; 6, commencement of cyst formation; 7, primary division of the nucleus within the cyst; 8, incomplete cell-division and formation of chromides; 9, formation by the chromides of two pairing nuclei; 10, formation of two reduced nuclei by each pairing nucleus; 11, division of each of the reduced nuclei into a free nucleus (male) and a stationary nucleus (female); 12, two nuclear stage formed by the merging of one free and one stationary nucleus; 13, adult cyst containing eight daughter-nuclei formed by the repeated division of the new nucleus; 14, the adult cyst after reaching the intestine of the host, liberating the eight young amœbæ formed during sporogony.

each mass of chromatin being surrounded by a little cytoplasm, thus forming a young amœba. During this process some of the chromatin is extruded from the parasite while some remains as a residual mass or masses.

Reproduction Within a Cyst.—When conditions are unfavorable for the vegetative existence of amœbæ they undergo encystment and reproduction occurs within the cysts. The method of reproduction within a cyst varies in different species and occurs in two different ways: either by the primitive mitosis of the nucleus, followed by autogamous fertilization; or by the nuclear chromatin becoming dispersed throughout the cytoplasm followed by the formation of minute buds containing chromidia which are separated from the parent body and form spores by the secretion of a resisting membrane. In this condition they are incapable of undergoing further development until surrounded by conditions favorable to vegetative life. These methods of reproduction will be considered more fully in the discussion of individual amœbæ.

Conjugation.—It has been demonstrated that in certain species of amœbæ conjugation between two individuals not infrequently occurs. I have observed it repeatedly in *Entamœba coli,* and rarely in *Entamœba histolytica* and *Entamœba tetragena.*

It is uncertain just what occurs during the conjugation of organisms of this class, but it is probable that it leads to a rejuvenescence of the vital processes rather than that it is a process of fertilization.

Resistance to Physical Conditions and Chemical Agencies.—Some species of free-living amœbæ are able to withstand a considerable degree of heat and cold, but the parasitic amœbæ of man become motionless, as a rule, after exposure for an hour to temperatures below 75° F. It has been suggested by some authorities that the species living in man can be best distinguished from free-living forms by their lesser resistance to heat and cold, the free-living forms remaining motile when exposed to great variations in temperature, while the parasitic amœbæ are only motile at a temperature near that of the human body. The writer does not believe that this method of differentiation is of much value, as he has frequently observed amœbæ, belonging to the genus *Entamœba,* moving about on a microscopic slide at a temperature far below that to which they are accustomed in the human intestine. Harris and Musgrave have shown that a temperature of 0° F. is not sufficient to kill some amœbæ, but it should be remembered that only the encysted forms are capable of withstanding such low temperatures.

As regards the resistance of these organisms to

chemical agencies it may be stated that a large number of such substances are capable of destroying them. A 1 to 800 solution of quinine sulphate will kill the amœbæ of man within a few minutes, while weak solutions of hydrogen dioxide, permanganate of potassium, sulphate of copper, nitrate of silver, argyrol, and dilute acids are efficient agents in the destruction of this class of organisms. Unfortunately, the parasitic amœbæ are not as easily destroyed when present in the intestine as they are upon a microscopic slide, as the pathogenic forms penetrate deep into the tissues where chemical solutions cannot reach them. Our knowledge is incomplete as regards the effect of chemicals upon the amœbæ of man, as most of the experimental work has been done with cultures of free-living species. In a recent paper Vedder gives in detail some very important experiments in which he found that ipecac, not de-emetized, killed a cultural amœba in dilutions as high as 1–50,000 in 24 hours; emetin, 1–100,000; quinine, 1–200,000; and silver nitrate 1–300,000. How far his results apply to the parasitic amœbæ of man is undetermined.

Many other chemicals, strong electrical currents, and the Roentgen rays are capable of destroying amœbæ, but none of these agents have proven of much service in the treatment of amœbic dysentery.

III.

CLASSIFICATION AND NOMENCLATURE.

From the time of the first description of a parasitic amœba of man the classification and nomenclature of these organisms has occasioned much confusion and difficulty. This is due to the great resemblance in the general morphology of the parasites belonging to the various species and to the difficulty and labor of studying their life cycle. While for years all authorities have agreed that these organisms belong to the Protozoa, almost every writer, until recently, has differed in his classification or in his conception of the biological history of the amœbæ associated with dysentery, and it was not until Schaudinn's observations were published that a really satisfactory classification of these parasites was possible.

However, it is probable that the present accepted classification may be found more or less erroneous as our knowledge increases, and it should be understood that *in any of the amœbæ in which the life cycle has not been thoroughly worked out, classification can only be provisional.* There are a few well-studied species occurring in man in which the present

classification may be said to be final, but other species have been described and classified which are still in doubt and in which the present classification may or may not be correct.

The *Amœbina* are divided by zoölogists into the *Reticulosa,* having filamentous pseudopodia; and the *Lobosa,* having lobose pseudopodia; the latter are divided into the *Gymnamœba,* in which the protoplasm is naked; and the *Testacea,* in which the protoplasm is surrounded by a shell.

The parasitic amœbæ of man belong to the *Gymnamœba,* or naked amœbæ having lobose pseudopodia, and are placed in the genus *Entamœba* created by Casagrandi and Barbagallo. All free-living forms are placed in the genus *Amœba* and are differentiated from the members of the genus *Entamœba* by the presence of a contractile vacuole and other morphological characteristics, and their inability to exist as parasites within man.

In addition to the two genera mentioned, we must include the genus *Paramœba,* which contains a marine amœba, *Paramœba eilhardi,* and the parasitic form known as *Paramœba hominis.*

The absence of amœbæ in numerous typical cases of dysentery, and their presence in health and diseases other than dysentery, has gradually led to the grouping of students of the subject into three schools,

i.e., those believing that amœbæ are always harmless commensals, more numerous, it is true, in the feces of dysentery patients because of the more favorable environment; those believing that all amœbæ may be pathogenic if suitable conditions be present; and those believing that both harmless and pathogenic species may be present in the intestine of man.

Prior to Schaudinn's work several investigators had endeavored to establish a classification of these parasites based upon morphological differences, but without success so far as the general acceptance of any one classification.

In 1893, Quincke and Roos, as the result of their studies, divided the amœbæ of man into three species, as follows:

1. *Amœba intestini vulgaris,* 40 microns in diameter, with large granules, which was pathogenic for neither man nor cats.

2. *Amœba coli mitis,* similar in size and appearance to the preceding, but which was pathogenic for man alone.

3. *Amœba coli* (Loesch), about 25 microns in diameter, with a finely granular endoplasm, which produced dysentery in both man and cats.

This classification, which was based largely upon the results of animal experiments, was not conclusive, as from the description of the organisms it was evi-

dent that the authors were dealing with mixed infections with both harmless and pathogenic amœbæ. In addition, the names given to the first two species cannot stand, as they are not in accordance with the binominal system adopted in nomenclature.

In 1894 Kruse and Pasquale distinguished four varieties of amœbæ based entirely upon morphological characteristics: (1) a form presenting a very refractive protoplasm, found in normal feces; (2) a form showing irregular and small granules; (3) a form in which the endoplasm consisted largely of vacuoles; (4) a form in which the protoplasm was filled with foreign bodies. The two latter forms were found only in dysenteric feces.

It will at once be seen that the differences in these forms are so slight as to be of no scientific value as a basis of classification, and the fact that these authors described the form found in normal feces as being very refractive, is evidence that they confused the pathogenic and harmless species, although they recognized a pathogenic amœba, *Amœba dysenteriæ,* following Councilman and Lafleur who, in 1891, objected to *Amœba coli,* as a name for the amœba causing dysentery, and suggested the name "*Amœba dysenteriæ.*" The latter authors, while not attempting to differentiate species, expressed it as their opinion that under certain conditions both harmless

and pathogenic amœbæ may inhabit the intestine of man.

Celli and Fiocca, in 1895, described no less than six species of amœbæ infesting the intestine of man and named these organisms as follows: (1) *Amœba spinosa;* (2) *Amœba vermicularis;* (3) *Amœba diaphana;* (4) *Amœba reticularis;* (5) *Amœba lobosa,* variety *guttata;* (6) *Amœba lobosa,* variety *oblonga.* The names sufficiently describe the differential characteristics of these organisms, but they all differed in size, one species, *A. diaphana,* measuring only 0.5 to 2 microns in diameter. To one who has studied these organisms the description of an amœba measuring but 0.5 micron in diameter may well be viewed with suspicion, when we consider that such an organism would be but little larger than the smallest micrococcus. The largest amœbæ described by Celli and Fiocca did not exceed 10 microns in diameter, which proves that these authors were not dealing with true dysentery amœbæ and it is more than probable that many of the species described by them were really stages in the development of the intestinal flagellates.

Casagrandi and Barbagallo were the first investigators to accurately describe the species of amœba occurring in the feces of healthy individuals and now known as *Entamœba coli.* They established

for this amœba the genus *Entamœba* because they considered that these organisms differed both in morphology and life history from the fresh water amœbæ. They described very minutely the morphology and method of reproduction of this species and their investigations directed the attention of other workers to the occurrence of amœbæ in health and in diseases other than dysentery. However, they were not successful in differentiating the pathogenic and harmless amœbæ.

In 1900, Strong and Musgrave recognized two species of amœbæ occurring in their patients in Manila, and state that with the harmless amœba, which they call *Amœba coli*, they were never able to produce dysentery in cats; while with the pathogenic amœba, *Amœba dysenteriæ*, they had no difficulty in producing the disease in cats by the injection of feces or the contents of liver abscesses containing the living parasites.

While, according to Schaudinn, the first investigator to clearly identify and describe a pathogenic and non-pathogenic species of amœba in man was Jürgens, in 1902, I believe that to Strong and Musgrave really belongs the credit of making this observation, as their work was published in 1900, while that of Jürgens did not appear until 1902. There is but little doubt that Strong and Musgrave actually

differentiated the two amœbæ now known as *Entamœba coli* and *Entamœba histolytica,* but unfortunately they did not adopt the name *Entamœba* as a generic term.

In 1903, Schaudinn, following the work of Jürgens, definitely proved that two distinct species of amœbæ infested the intestine of man. His work rested not only upon differences in the morphology of the two parasites, but upon their entirely distinct methods of reproduction, which he studied very thoroughly. To the amœba occurring in the feces of normal individuals, or in those suffering from other diseases than dysentery, he gave the name *Entamœba coli,* while to those occurring in dysenteric feces and causing that disease, he gave the name *Entamœba histolytica.*

Stiles has written an exhaustive summary regarding the nomenclature of the amœbæ of man, in which he clearly states the nomenclatural situation, and concludes that for those who believe that there is but one species of amœba infecting man and that it is not congeneric with fresh water amœbæ, the correct name to use is *Entamœba coli,* not *Amœba coli,* for, as shown by Casagrandi and Barbagallo, the amœbæ of the human intestine differ from the fresh water amœbæ, to which the generic term "Chaos," later amended by Ehrenberg to "Amœba," was originally given; while for those who believe in a harmless and

a pathogenic species, the correct zoölogical names are *Entamœba coli* and *Entamœba histolytica* respectively.

In 1905, I suggested that as the name *Amœbæ dysenteriæ* had been given the pathogenic species by Councilman and Lafleur, the name *histolytica* should give way to *dysenteriæ,* but as Stiles has conclusively shown that the latter is merely a *synonym* of *coli,* it follows that it cannot be used to designate the pathogenic amœba, and therefore we must accept Schaudinn's name *Entamœba histolytica* for one of the species of amœbæ causing amœbic dysentery.

At the present time Schaudinn's classification has been accepted by most medical investigators and writers, and by all zoölogists with whose work I am acquainted. Musgrave and Clegg, almost alone of those who have had an extensive experience with amœbic dysentery, still decline to accept Schaudinn's classification, although they state "we do not at all question the multiplicity of both genera and species of amœbæ, both within and without the intestine of man." They also adhere to the name *Amœba coli,* although, as Stiles has shown, the proper generic term for the parasitic amœbæ of man is *Entamœba.*

The exact zoölogical position of the parasitic amœbæ of man, together with their specific names, may be tabulated as follows:

Phylum, Protozoa.
Sub-phylum, *Sarcodina.*
Class, *Rhizopoda.*
Sub-class, *Amœbina.*
Order, *Gymnamœbida.*
Genus, *Entamœba,* Casagrandi and Barbagallo, 1895.

Species, *Entamœba coli,* Losch, 1875, emend Schaudinn, 1903.
Entamœba histolytica, Schaudinn, 1903.
Entamœba buccalis, Prowazek, 1904.
Entamœba tetragena, Viereck, 1907.
Entamœba phagocytoides, Gauducheau, 1908.
Entamœba tropicalis, Lesage, 1908.
Entamœba minuta, Elmassian, 1909.
Entamœba nipponica, Koidzumi, 1909.
Entamœba kartulisi, Doflein, 1901.
Genus, *Paramœba,* Schaudinn, 1896.
Species, *Paramœba hominis,* Craig, 1906.

There are a number of other species of questionable value and it is possible that some of the species given above may have to be relinquished upon further study. Students of these parasites are becoming

more and more convinced that species differentiation should rest chiefly upon differences in the life cycle rather than upon mere morphological variations, and the writer believes that a species should not be described until at least the larger portion of its life cycle has been demonstrated.

The species which are still of uncertain value are the following:

Genus, *Entamœba.*
Species, *Entamœba undulans,* Castellani, 1905.
Amœba pulmonalis, Artault, 1900.
Amœba urogenitalis, Baelz, 1903.
Amœba miurai, Ijima, 1898.
Amœba gingivalis, Gros, 1848.
Amœba dentalis, Grassi, 1879.

IV.

TECHNIQUE.

THE parasitic amœbæ of man may be studied in the living condition, in stained smears of material containing them, and in stained sections of tissue. Each of these methods has its advantages, but for diagnosis and for the study of the vital activities of these parasites the examination of fresh material is the most satisfactory. It is never necessary for diagnostic purposes to stain amœbæ nor is it essential for the differentiation of species. It is very difficult to stain these organisms and even with the utmost care it will often be found that scores of preparations will have to be examined before a satisfactory one is obtained. As fixing and staining the organisms often cause them to assume appearances very unlike those observed in living specimens, it is my belief that stained preparations should never be depended upon for diagnosis except in the hands of an expert.

However, the use of stained smears is necessary in following out the nuclear changes during the life cycle of these organisms, for while in the living specimens such changes may be observed, they are much more definitely marked in the stained preparations.

The typical staining reaction of the chromatin makes it easy to follow the changes occurring in the nucleus during the various reproductive processes. For this purpose no other method gives as good results as the use of carefully stained specimens.

The great advantage of the use of stained sections of tissue lies in the knowledge which they furnish us regarding the exact relation of amœbæ to the pathological conditions present in the tissues examined. Thus in sections of the intestine from patients dying of amœbic dysentery the stained sections demonstrate the undoubted etiological relationship of the amœbæ to certain of the lesions present, and this is also true of sections of the liver in which abscesses due to these organisms are found. In no other way can we as well demonstrate the relationship of amœbæ to the lesions which they produce in man.

THE EXAMINATION OF LIVING AMŒBÆ.—A very small portion of a freshly passed stool should be placed upon a microscopic slide and covered with a cover glass, gentle pressure being used to spread the specimen. The material selected for examination should preferably be a drop of the liquid portion of the stool rather than solid particles. It is always well to give a saline cathartic before making an examination as this tends to wash the amœbæ from the intestinal walls. If present, a particle of mucus

or any blood-stained material should be examined as well. Most stools from amœbic dysentery cases contain gelatinous material which frequently contains numerous amœbæ, and such material should always be fully examined.

The feces should be examined as quickly as possible after they have been passed as the amœbæ are much more easily recognized when they are motile. No disinfectant should be used in stools which are to be examined for amœbæ, nor should urine be mixed with the stools. In temperate regions, especially in the winter, the receptacle used in collecting the specimen should be warmed, but care should be taken if water is used for this purpose, that it be boiled, as otherwise water amœbæ might be mistaken for parasitic amœbæ, having reached the feces in this manner.

Unless the reproductive cycle of an organism is to be studied the warm-stage is not necessary, but it should always be used in research work upon these parasites, as in no other way can the various stages in growth and reproduction be thoroughly studied. When extensive studies of the vital activities of amœbæ are to be undertaken, an incubator, designed to contain the microscope, will be found to be a great convenience.

Although it is generally stated that entamœbæ

become motionless at room temperature after an hour or so, it is not unusual to observe motility in these parasites in specimens of feces which have been collected for several hours. If the amœbæ are not motile the gentle warming of the slide will often restore motility, provided degeneration of the parasites has not occurred. Even when the amœbæ are motionless it is not difficult, for one accustomed to observing them, to distinguish them from other bodies occurring in the feces. Many observers claim that a diagnosis of the presence of amœbæ in feces should never be made unless the organisms be motile. While this is valuable advice for the novice in this line of work, it is certainly true that by one who has studied these organisms, they can be easily recognized when motionless, provided the feces has not been kept so long as to have led to degenerative changes in the amœbæ.

In making fresh preparations it is always well to dilute a loopful of the stool with normal salt solution or distilled water. One of the most frequent mistakes made in examining such preparations is the use of too thick a preparation. One should not be satisfied with the examination of a single slide, but should thoroughly examine at least six or eight preparations before a negative result is reported.

The hanging drop method is often valuable in

the examination of these organisms, a small drop of the stool being placed in the centre of a cover glass which is then inverted upon a hollow ground slide and ringed with vaseline. If a film preparation is used it should always be ringed with vaseline, for unless this is done evaporation occurs and the preparation becomes useless.

The preparation should be examined with a one-sixth inch lens and a one- or two-inch eyepiece. For the finer details regarding the structure of the cytoplasm and the nucleus, as well as the reproductive changes, it is necessary to use the one-twelfth inch oil immersion objective.

Neutral Red.—The use of a solution of 1/10,000 of neutral red is often of great service in those cases in which the amœbæ are few in number and for the study of structural details. This solution is very quickly absorbed by the amœbæ, coloring them pink or red, and does not interfere with their movements if it is not used in too strong a dilution. It is a most useful method in distinguishing between parasitic amœbæ and leucocytes, as the latter do not stain with this substance. In specimens in which the amœbæ are in scant numbers they are easily distinguished by the reddish color given them by the neutral red, as other cells occurring in feces are not colored distinctly by this dye. The dilution should be made with normal salt solution.

METHODS OF STAINING MATERIAL CONTAINING AMŒBÆ.—Owing to the very delicate structure of amœbæ any method of fixation and staining which may be adopted undoubtedly causes some change in size and morphology and one's object should be to use methods which will obviate, as far as possible, such artificial changes. For the best results in the study of the morphology of the nucleus of amœbæ, as well as other Protozoa, and more especially the changes occurring in this structure during multiplication, fixation of the wet specimen with osmic acid vapor or sublimate alcohol, followed by staining while the specimen is still wet, is essential, although air-dried specimens, which are afterward stained, are sufficient for ordinary work. I was able to confirm all the essential details of Schaudinn's work upon *Entamœba histolytica* and *Entamœba coli* in air-dried smears of material containing these parasites, stained with Wright's modification of the Romanowsky stain, although only after months of study and the examination of many thousands of preparations. Had the wet fixing and staining methods been used at that time much labor would have been saved and the mitotic division of the nucleus would have been demonstrated. In making stained preparations it is first necessary to fix upon a slide or cover glass the material to be examined. For this purpose numerous fixing mixtures

have been elaborated. Among the most important are the following:

Osmic Acid.—This mixture consists of two parts of osmic acid in 100 parts of a 1 per cent. chromic acid solution, or a 4 per cent. solution of osmic acid to which a certain amount of glacial acetic acid is added at the time of fixation. (1 drop of glacial acetic acid to 20 drops of the 4 per cent. solution of osmic acid.)

It should be remembered that osmic acid is a strong irritant of the mucous membranes and care should be taken not to inhale the fumes or allow them to come in contact with the eyes. The sealed tube containing the acid should be broken in a glass stoppered bottle containing the desired amount of water or other solution.

Absolute Alcohol.—When dry cover glass preparations are to be examined they may be fixed by immersing them in absolute alcohol for from 2 to 5 minutes.

Alcoholic Solution of Mercuric Chloride.—The mercuric chloride used in this mixture is obtained by dissolving perchloride of mercury in boiling normal saline solution in such proportions that a few crystals of perchloride of mercury are deposited in the vessel after boiling. In other words, a saturated solution of perchloride of mercury in boiling saline solution is to be obtained.

The mixture is made by adding one part of absolute alcohol to 2 parts of the saturated solution of mercuric chloride and it should be used warm, the preparations being immersed for from 2 to 5 minutes. They are then washed in 50 per cent. alcohol, and 70 per cent. alcohol, and then placed in 70 per cent. alcohol to which enough tincture of iodine has been added to give a port wine color. They are allowed to remain in this mixture until they turn a pale yellow, when they are rinsed in 70 per cent. alcohol, and hardened in 80 per cent. alcohol for at least a quarter of an hour.

Acetic Acid Solution of Picric Acid and Mercurial Sublimate.—This mixture consists of equal parts of a saturated solution of mercuric chloride prepared as above; picric acid, 1 per cent. in distilled water; and a ½ to 1 per cent. solution of glacial acetic acid. This method is most useful in fixing portions of tissue containing amœbæ. The tissue should be left in the mixture over night and then washed in 50 per cent. and 70 per cent. alcohol.

Sublimate Acetic Acid Mixture.—This mixture consists of 95 parts of a saturated solution of sublimate of mercury in distilled water and 5 parts of glacial acetic acid. The cover glasses containing the material to be examined are placed directly in this mixture while wet or after a preliminary fixation with osmic acid vapor.

Cover glass preparations may be fixed either wet or dry. If one desires to study the minute morphology of amœbæ it is best to fix the preparations wet, although carefully prepared dry specimens often give good results, and the writer has found that dry preparations are just as useful for ordinary work. If the specimens are to be fixed wet the following procedure is to be recommended:

The stool containing the amœbæ is spread in as even and thin a layer as possible upon the cover glass, which is then placed with the material downwards in a dish containing the fixing solution. While some of the material will dissolve in the solution, it will be found that most of the amœbæ are retained upon the cover glass. After fixing, the preparations are rinsed in alcohol (50 per cent.) and hardened with 70 or 80 per cent. alcohol.

A quick and efficient method of wet fixation is by exposure to osmic acid vapor, the osmic acid solution already described being used for this purpose. This method is most useful if Giemsa's or Wright's staining methods are used and it is desired to avoid artificial changes in the structure of the amœbæ. The technique of fixation by this method is as follows:

A cell, slightly smaller in diameter than the cover glass containing the material to be examined, and from one-half to three-quarters of an inch deep, is

hollowed out in a block of hard paraffin. The osmic acid solution is placed within this cell, the latter being filled to within a short distance of the surface. The edge of the paraffin cell is then smeared with vaseline and the cover glass then placed preparation-side downward over the cell, and pressed into the vaseline, thus making an air-tight junction. After exposure to the vapor for from 30 seconds to 1 minute the preparation is at once placed in absolute alcohol, and afterward stained, and mounted in acid-free Canada balsam. At no stage of the process should the specimen be allowed to dry if the best cytological results are desired. The osmic acid mixture preferred consists of 20 parts of a 4 per cent. osmic acid solution and 1 part of glacial acetic acid.

Various *staining methods* may be used in coloring the parasitic amœbæ of man. Among the most simple are carbol-fuchsin, methylene blue, gentian violet and hæmatoxylin. The latter is most valuable in demonstrating these parasites and Delafield's hæmatoxylin gives the best results. It should be well diluted with distilled water and the preparations should be left in it over night, after which they are washed in running water, and, if well stained, are rinsed in 50 per cent., 70 per cent., 90 per cent., and absolute alcohol, and mounted in Canada balsam, which is free from acid. If the specimens are too deeply stained they

may be decolorized in 70 per cent. alcohol containing a few drops of hydrochloric acid, the process being watched under the microscope until the parasites appear a pale bluish black. They are then placed in a solution consisting of 1 drop of ammonia to 100 c.c. of 70 per cent. alcohol until the color turns blue, when they are rinsed in 70 per cent. alcohol and mounted. If desired, the cytoplasm of the amœbæ may be stained by eosin after they have been stained with hæmatoxylin which, of course, only colors the nucleus.

If carbol-fuchsin, methylene blue, or gentian violet is used for staining, the fixed preparations are placed in the staining solution for a few moments, washed in 70 per cent. alcohol, and mounted. These stains are of very little service in the study of these parasites.

The Iron Hæmatoxylin Method of Heidenhain.—This is a most valuable method for studying structural details. If it is used the specimens must be fixed wet with alcoholic solution of mercuric chloride or with osmic acid vapor. The following solutions are used in this method:

1. A 2.5 per cent. watery solution of iron-alum (Ferrous ammonium sulphate).

2. A 0.5 to 1 per cent. watery solution of hæmatoxylin, which should be at least four weeks old.

Cover-glass preparations or tissues, previously rinsed in distilled water after fixing, should be placed in solution No. 1 over night, after which they are rinsed in distilled water and immersed in solution No. 2 for from 6 to 24 hours and in the case of sections as long as 36 hours. They are then rinsed in distilled water and decolorized in solution No. 1, this process being controlled by observation under the microscope, until the nucleus is well differentiated, the chromatin staining a deep blue-black and the cytoplasm a light gray; the preparations are then washed in running water for half an hour and rinsed in 50 per cent., 70 per cent., 90 per cent. and absolute alcohol, cleared in xylol, and mounted in Canada balsam.

This method requires considerable experience in order to obtain the best results, but may be said to be the best method we possess for the study of the structural details of amœbæ.

The Giemsa Stain.—Very beautiful specimens may be obtained by this method. Its chief objection is that the method of preparation is so complicated and requires so much care that it can only be used in a well equipped laboratory. However, a reliable Giemsa stain may be obtained from Grubler. The following is the method of preparation of this stain and of its use in staining amœbæ:

The utmost care must be taken that everything used in preparing this stain, and using it, is chemically clean, and that none of the dishes or implements used are moist and that they be sterilized before use.

The *stock solution* consists of Azure II-eosin, 3 grams, and Azure II, 0.8 gram. These are dissolved, by constantly shaking, in 250 c.c. of pure glycerine at a temperature of 60 degrees C. After solution is complete, 250 c.c. of absolute methyl alcohol (Kaulbaum I) previously heated to 60 degrees C. is added. This mixture is well shaken, allowed to stand at room temperature for 24 hours, and then filtered into a chemically clean, sterilized, air-tight bottle. During the filtration the funnel must be covered in order to protect the hydroscopic fluid from moisture and the bottle containing the stock solution should be kept in a dark place.

The method of using this stain is as follows:

Thin, even smears of the material containing the amœbæ are made upon cover slips and fixed while wet in osmic acid vapor or sublimate alcohol, and stained with the following solution:

Stock solution ....10 drops.
Distilled water ....10 c.c.
1 per cent potass. carb. solution, 1 or 2 drops to each 10 c.c. of the above mixture.

This staining solution is allowed to act for from 30 minutes to 6 hours, although fairly good prepara-

tions may be obtained after staining for from 10 to 15 minutes. The stained smears are finally washed in running distilled water, the excess of stain removed with alcohol, cleared with xylol, and mounted in acid-free Canada balsam. A good Giemsa stain may be obtained from Grubler and is used in the proportion of 1 drop of the stain to 1 c.c. of distilled water. With this wet-fixed specimens should be stained for from 2 to 6 hours, and cleared and mounted as just described. Fair results are obtained with this stain in air-dried preparations.

Wright's Method.—The following method I have found very satisfactory in staining parasitic amœbæ and with it I have been able to follow the entire cycle of development of *Entamœba coli* and *Entamœba histolytica,* as described by Schaudinn. It should be remembered, however, that one has to examine many preparations before this can be accomplished and that the majority of air-dried preparations are almost useless for the study of minute cytological details.

The following chemicals are used in preparing Wright's stain:

1. Methylene blue (Grubler's).
2. Eosin. Yellow. Water soluble (Grubler's).
3. Methylic alcohol (Merck's reagent).

Method of Preparation.—The stain is prepared as follows: Add 0.5 gm. of sodium bicarbonate to 100 c.c. of distilled water, dissolve thoroughly, and

add 1 gm. of methylene blue (Grubler); heat the mixture for one hour in an Arnold sterilizer or other steam sterilizer, after the steam is up. After heating allow the solution to cool. Make a 1 to 1,000 solution of yellow aqueous eosin (Grubler) and add this, while stirring, to the cooled methylene blue solution, in about the proportion of 500 c.c. of the eosin solution to 75 c.c. of the methylene blue solution. This should be done in a white dish of some kind, in order that the precipitate that forms as the eosin solution is added may be easily seen. The proportion of eosin solution added to the methylene blue solution will vary in different batches of the stain, but the eosin solution should always be added until a well-marked precipitate follows after stirring. A marked metallic scum will appear upon the surface of the mixture at the time the eosin solution is in sufficient quantity and this may be used as an indicator, but if no precipitate is present when the metallic scum appears the eosin solution should be added until it appears. The mixture should then be allowed to stand for fifteen or twenty minutes, and then filtered through one small filter-paper, the precipitate saved, dried in a hot-air oven, removed from the filter-paper, and used in making the staining solution. The precipitate, which is a fine powder, greenish in color, will keep well and can be used as stock material for months.

The staining solution is prepared as follows: Take 0.3 gm. of the powdered precipitate and add it to 100 c.c. of pure methylic alcohol (Merck's reagent), filter, and to 80 c.c. of the filtrate add 20 c.c. of the methylic alcohol, or if more than 80 c.c. be left after filtration, enough to bring the entire amount up to 100 c.c. The staining solution is now ready for use and will keep unimpaired for weeks.

To stain, add a few drops of the staining solution to the preparation of feces without preliminary fixation, or previously fixed, while wet, with osmic acid vapor followed by alcohol, and let stand for five minutes; then add enough distilled water to cause a slight metallic scum to form on the surface of the preparation; let stand for from 10 minutes to an hour and wash thoroughly in running distilled water. If stained too deeply, wash in alcohol and xylol. If it is desired to preserve the specimens, mount in Canada balsam. The best results are obtained with wet-fixed preparations, but carefully stained air-dried specimens give good results for ordinary work.

Other methods of staining the amœbæ in the feces, or in the pus from liver abscesses, have been recommended, but none of them will be found as satisfactory as those which have been described.

TECHNIQUE OF STAINING SECTIONS OF TISSUE CONTAINING AMŒBÆ.—Sections of the infected intestine or of the walls of liver abscesses are most use-

ful in demonstrating the relation that the amœbæ bear to the lesions which are present. Various staining methods are used for this purpose, the most useful of which is probably Mallory's stain, which differentiates the amœbæ in tissues in a very satisfactory manner.

Mallory's Method.—Small pieces of tissue are hardened in various strengths of alcohol, as is usual, and imbedded in paraffin. As thin sections as possible should be cut and stained for from 3 to 5 minutes in a saturated aqueous solution of thionin; differentiated in a 2 per cent. aqueous solution of oxalic acid for ½ to 1 minute; washed in water; dehydrated in absolute alcohol; cleared in xylol and mounted in xylol balsam.

With this method the nuclei of the amœbæ stain a brownish red, while the nuclei of other cells stain blue.

Safranin.—A good stain for demonstrating the amœbæ in tissue is safranin. The tissues may be hardened in alcohol or in Flemming's or Zenker's fluids, and then imbedded in paraffin. The following solution of safranin is used for staining: Equal parts of a saturated aqueous solution of "safranin 0 soluble in water" and a saturated alcoholic solution of "safranin soluble in alcohol."

The sections are stained in this mixture for from 6 to 24 hours, washed in water, and differentiated in

absolute alcohol, after which they are cleared in xylol and mounted in xylol balsam.

Eosin and Methylene Blue Stain.—This stain gives good results although it does not differentiate the amœbæ from the cells of the tissues. However, to one accustomed to the examination of such tissues there is no difficulty experienced in recognizing the amœbæ. If this stain is used the sections should be fixed in Zenker's fluid and imbedded in paraffin.

The method of staining is as follows: Paraffin sections are stained in 10 per cent. aqueous solution of eosin for from 15 to 30 minutes; washed in water until the excess of eosin is removed; and stained in Unna's alkaline methylene blue solution diluted 5 times with water, for from 10 to 15 minutes. The Unna solution consists of the following:

Methylene blue (Grubler)	1.0 gram.
Carbonate of potass.	1.0 gram.
Distilled water.	100.0 c.c.

After remaining in this solution for the required time the sections are washed in water and differentiated and dehydrated in 95 per cent. alcohol. This procedure should be controlled under the microscope and should be continued until a pink color has returned to the section and the nuclei of the cells appear a deep blue; absolute alcohol is then used to complete the dehydration, the sections are cleared in xylol, and mounted in xylol balsam. Care should be taken

that during the first staining with eosin a deep red color be obtained as otherwise it will all be removed by the methylene blue solution.

The Hæmatoxylin-Eosin Stain.—Sections may be stained by this method and give fairly good results. They should first be fixed in Zenker's solution and then imbedded in paraffin. The solution of hæmatoxylin used is that known as Delafield's and is prepared as follows:

Four grams of hæmatoxylin crystals (Grubler's) are dissolved in 25 c.c. of 95 per cent. alcohol and to this is added 400 c.c. of a saturated aqueous solution of ammonia-alum, the mixture then being allowed to stand in an unstoppered bottle exposed to the light and air for four days. It is then filtered and to the filtrate is added 100 c.c. of glycerine and the same amount of 95 per cent. alcohol. This solution is allowed to stand in the light until it has a deep purple tinge, when it is filtered and stored in a tightly stoppered bottle. If the purple tinge is lost the solution is no longer suitable for staining.

In using the stain for section work the sections are stained in it for 30 minutes to an hour, or more, and are then washed in several changes of water or in running tap water for from 10 to 30 minutes. After they are thoroughly washed, an aqueous solution of eosin of ½ per cent. strength is used for a

contrast stain, after which the sections are dehydrated in 95 per cent. alcohol, cleared in oleum origani cretici, and mounted in Canada balsam. As good results are obtained by adding a few drops of the hæmatoxylin solution to a small dish full of water containing the sections and allowing them to stay in this solution over night, after which the contrast stain is used as described.

Heidenhain's Iron-hæmatoxylin Method.—Sections fixed with Zenker's fluid and not over 6 microns in thickness may be stained by the Heidenhain method in the same manner as heretofore described for staining smears of material containing amœbæ.

A great deal of care is required in staining sections containing amœbæ if the best results are to be obtained, and sometimes many preparations have to be stained before typical ones are secured. This is true of any method which may be adopted, although the writer believes that the Mallory method is the easiest of application and gives the most uniformly satisfactory results.

For the diagnosis of amœbæ in the stools staining methods should never be resorted to, as the examination of fresh specimens is altogether superior and safer. The stained preparations are only used in the study of the morphological character and the methods of reproduction of these organisms.

V.

THE CULTIVATION OF PARASITIC AMŒBÆ.

MANY authorities have claimed to have cultivated the parasitic amœbæ of man, while others have insisted that such cultivation has not been accomplished. Anyone studying the literature is struck with the discordant results obtained by different observers, and I believe that it is now generally accepted by zoölogists, who have thoroughly studied these organisms, that none of the parasitic amœbæ of man have been cultivated. While it is not at all difficult to cultivate free-living forms, it is very doubtful, to say the least, if any of the amœbæ which have become parasitic can be cultivated by the methods at present in vogue.

It is admitted by all that amœbæ have been cultivated from the feces of patients suffering from dysentery, but it cannot be admitted that these amœbæ are parasitic forms; in reality they are free-living amœbæ which have reached the intestine in food or drink, and have simply passed through it in an encysted condition. If cultures be made from the feces containing these free-living species they will develop if suitable bacteria

be present and it is this fact which has led to the mistaken idea that the parasitic amœbæ are easily cultivated.

Regarding this subject Braun and Lühe, in their latest work upon parasitology, published in 1910, say: "It must be borne in mind, however, that not *all* amœbæ can be cultivated upon solid media, *and that the varieties which are parasitic in mammals have never been successfully grown outside of the tissues of their host.*" This statement is concurred in by the writer, for I have never been able to obtain any amœbæ upon cultural material which resembled those parasitic in man, nor have I ever seen amœbæ cultivated by others which agreed in either their morphology or their methods of reproduction with the parasitic species occurring in the human host. All cultivated amœbæ that I have observed possess a contractile vacuole and otherwise resemble in their morphology the common free-living species. The successful production of dysenteric lesions in animals by the use of cultures of amœbæ obtained from dysenteric feces proves nothing, as the material could easily be contaminated by the minute spores of *Entamœba histolytica,* or encysted forms of other pathogenic species, or by bacteria capable of causing dysentery.

In regions where free-living amœbæ are common, as in the tropics, it is not at all difficult to cultivate

amœbæ from the feces of man either in health or disease, but in temperate regions, where free-living forms are comparatively rare, it is but seldom that one is successful in cultivating such organisms from the feces. Numerous attempts have been made to cultivate amœbæ at this laboratory from patients whose stools contained *Entamœba histolytica* and *Entamœba tetragena,* as well as *Entamœba coli,* and in not a single instance have cultures been obtained of any amœbæ, in spite of the fact that the same methods were used that had proven successful in the Philippine Islands, and that the same observers had secured amœba cultures in those Islands without trouble. However, it is not difficult to explain these results. In the Philippines almost all drinking water, as well as salad vegetables, are infested with free-living amœbæ, and it naturally follows that such forms are continually passing through the intestinal canal of man, and may be obtained upon suitable culture media from the infected feces; in Washington, on the other hand, free-living species of amœbæ are comparatively rare, and it follows that attempts to cultivate amœbæ from the feces generally result in failure, as the parasitic species do not develop upon the culture media at present in use. To my mind the failure to cultivate amœbæ from dysentery patients in certain regions, although the same methods which are suc-

cessful in other regions are employed, is sufficient proof that the parasitic amœbæ of man have not been cultivated. In no other way can we explain the successful results attending the cultivation of amœbæ in the tropics and the unsuccessful results in temperate regions even though material be used from patients heavily infected with *Entamœba histolytica,* or *Entamœba coli.*

A review of the literature on the subject and of the methods which have been used in cultivating amœbæ is here given. I do not believe that any of the methods recommended for cultivation have been successful so far as the parasitic species of man are concerned, but modifications of them may prove of service in further research work upon these parasites, while the study of cultures of free-living species is of service in comparative morphology.

Cunningham in 1879, and Grassi in 1882, claimed to have been able to cultivate dysentery amœbæ and stated that such cultures produced dysentery in cats.

In 1890, Kartulis attempted to cultivate amœbæ from the intestinal discharges of cases of dysentery occurring in Egypt and stated that he obtained pure cultures in sterile straw infusion and in feces diluted with alkaline bouillon. Both his observations and those of Cunningham and Grassi are now discredited so far as the cultivation of the parasitic species of man is concerned.

In 1897, Frosch demonstrated that it was possible to grow free-living amœbæ upon culture media, provided bacteria were present at the same time, and that unless such bacteria were present it was impossible to carry the organisms from one culture to another. In the same year Casagrandi and Barbagallo confirmed these observations, but considered that the association of the amœbæ and bacteria was simply an accidental one. Tsujitani, in 1898, cultivated amœbæ in association with cholera vibrios, and by heating the cultures at 60 degrees C. he killed the vibrios, thus obtaining a pure culture of the amœbæ. He found, however, that while the encysted amœbæ upon the cultures were capable of motility and growth, multiplication did not occur when they were transferred to sterile culture media. His observations, together with those of Zaubitzer, Mouton, and Schardinger, definitely proved that it is impossible to transplant pure cultures of amœbæ free from all bacteria.

The most extensive work done upon the cultivation of the parasitic amœbæ of man is that of Musgrave and Clegg. In 1904, these investigators published a monograph upon this subject in which a complete review was given of the literature, together with their methods and results. They claim to have been able to cultivate dysentery amœbæ in symbiosis

with pure cultures of bacteria and to have produced in monkeys typical symptoms of the disease by the use of such cultures. While there can be no question that they were able to cultivate amœbæ from the feces of dysentery cases it is still uncertain just what species were obtained in this way, and while lesions were undoubtedly produced by the mixed cultures of amœbæ and bacteria, the authors could not, with their methods, be sure of excluding the spores of *Entamœba histolytica,* or the encysted forms of other amœbæ pathogenic to the animals used in their experiments. As they did not accept Schaudinn's classification, they made no differentiation between pathogenic and free-living amœbæ, and many of their photomicrographs of the organisms from cultures prove that they were dealing with ordinary free-living species.

Walker, in 1908, following and amplifying the methods of Musgrave and Clegg, claims to have successfully cultivated numerous amœbæ, including parasitic species, but his description of the life cycle of the parasitic forms does not agree with that of Schaudinn, Doflein, Lühe, Viereck, and many others who have thoroughly studied the methods of reproduction of amœbæ found both in health and disease. It is more than probable that the amœbæ cultivated by Musgrave and Clegg, and by Walker, were free-living species occurring accidentally in the feces.

TECHNIQUE OF CULTIVATION.—A large number of culture media have been recommended for the cultivation of amœbæ. Kartulis recommended a solution of ordinary bouillon and sterilized infusions of hay; Ogata used a 2½ per cent. solution of grape sugar in sterilized water; Vivaldi used sterilized straw infusion, and Miller was able to grow the organisms in a 1/5 per cent. solution of milk, in water, in hay infusions, and in dilute bouillon. A number of observers have used solid media, such as agar, alkaline potatoes, egg albumin, and 5 per cent. of *Fucus crispus* in alkaline albumin. Among other media which have been used may be mentioned Gensen's, composed of barley sprouts cooked in water, filtered, rendered alkaline, and mixed with sugar, after which it was sterilized by the fractional method; Zaubitzer's, consisting of solutions containing varying amounts of somatose or agar containing from 1 to 2 per cent. somatose; and Casagrandi and Barbagallo's, consisting of the sterile white of egg containing bicarbonate of soda.

While it is true that free-living amœbæ may be cultivated upon almost all of these media the one which has given the most satisfactory results is that devised by Musgrave and Clegg, the formula of which is as follows:

Agar	20.0 grams.
Sodium chloride	0.3–0.5 gram.
Extract of beef	0.3–0.5 gram.
Distilled water	1000. c.c.

This is prepared exactly as is ordinary nutrient agar and is made 1 per cent. alkaline, using phenolphthalein as an indicator. Upon this medium numerous species of free-living amœbæ develop, provided suitable bacteria are present in symbiosis. Musgrave and Clegg have determined that many amœbæ are very selective in regard to the bacteria with which they will develop, and they regard failures to grow amœbæ as largely due to the absence of a suitable symbiotic bacterium.

Because of this selective action of the amœbæ it is possible to grow them in pure culture with a single species of bacterium and these cultures are known as "pure mixed cultures of amœbæ." Of the bacteria which have been found most suitable for cultivation with amœbæ may be mentioned *Bacillus coli, Vibrio choleræ, Bacillus typhosus, Staphylococcus pyogenes aureus* and such non-pathogenic organisms as *Bacillus fluorescens* and *Bacillus rubra.*

It is a significant fact that in most of the experiments resulting in the production of pathogenic lesions by the use of cultures of amœbæ, the bacteria grown in symbiosis with the amœbæ have been pathogenic species. For this reason the results must be regarded

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as doubtful as one can hardly draw conclusions regarding the pathogenicity of the amœbæ from the results of injection into animals of these mixed cultures containing both amœbæ and pathogenic bacteria.

Cultures from Water.—The method recommended by Musgrave and Clegg for securing cultures of amœbæ from water is the following:

From 100 to 500 c.c. of the water to be examined is collected in a sterile flask and ½ to 1 c.c. of 1 per cent. alkaline bouillon is added to each 100 c.c. of the sample. The flask is stoppered with cotton, and placed in the dark for from 48 to 72 hours, and at the end of this time a loopful of the water from the surface is examined for amœbæ. A loopful is then spread over the surface of the medium used, preferably that described above, which has been poured into a Petri dish. The plate should be examined at the end of 24 hours, and in many instances amœbæ will be found at that time, but sometimes several days elapse before the cultures develop. It is not necessary to use a symbiotic bacterium, as the amœbæ will find suitable bacteria in the water.

Cultures from Feces.—For the cultivation of amœbæ from feces the following method is recommended by Musgrave and Clegg:

The surface of the special culture medium, contained in Petri dishes, is smeared with a culture of

Bacillus coli or some other bacterium with which amœbæ grow well, and the feces is then smeared in concentric circles upon the surface of the medium or in streaks across it. The plates are then kept at room temperature and examined upon successive days for at least a week. The amœbæ can be easily seen upon the plates, as they wander from the material in the spread out into the surrounding medium and the course that they take can often be followed by the development of colonies of the bacteria, which they carry with them.

Both Musgrave and Walker state that the development of the parasitic species occurs more readily at room temperature than in the incubator at 37° C. As such species are normally subjected to the temperature of the body, it is difficult to understand why they should grow best under artificial conditions at a lower temperature. This is certainly not true of any other protozoön and suggests that all their cultivated amœbæ were really free-living forms. In every instance where cultural forms of the protozoa have been obtained, in which growth occurs at a lower temperature than that of the host, such parasites have depended for transmission upon some insect having a low body temperature. There is no evidence that the parasitic amœbæ undergo any development in, or are transmitted by, an insect, and I consider that

the failure of so-called parasitic amœbæ to develop upon culture media at the body temperature is additional proof of the free-living nature of the amœbæ which have been cultivated. However, even free-living amœbæ may be cultivated at 37° C. without much difficulty.

A simple and easy method of securing cultures of amœbæ from feces consists in mixing with the material to be examined an emulsion of the bacterium to be used in symbiosis and streaking this mixture across the surface of the culture medium contained in Petri dishes.

To secure a culture of a single amœba in symbiosis with bacteria the culture plates are carefully examined and a single amœba is located. In order to remove this organism from the plate, Musgrave's method may be employed, which consists in lowering a low power objective until it just touches the medium where the amœba lies, and then quickly raising it, thus picking up the amœba, which can then be transferred to a new plate; or a single organism is located and with a fine glass capillary pipette is removed from the surface of the plate, and placed upon new media. Walker marks the situation of the single amœba with a wax pencil on the bottom of the Petri dish, using a V-shape mark, the apex of which just encloses the amœba, the arms of the V extending

away from the streak of bacterial growth; the plate is then turned right side up, the cover removed, and with carbolated vaseline the V-shape mark is traced on the surface of the medium. In this manner the single amœba is isolated and can be transferred, after multiplication, to a new plate. It is well to cover the surface of the medium outside the arms of the V with a little additional vaseline.

The Cover-glass Method of Cultivation.—This very ingenious method of cultivating amœbæ was devised by Walker and is thus described by him:

" It is based on the principle of Koch's original plate culture for bacteria, is indeed a miniature Koch's plate culture made on a cover-glass and inverted over a concave slide. For this reason I have called such culture a ' hanging plate culture.' This culture is prepared as follows: A thin seven-eighths-inch cover glass is flamed and placed under a flamed watch glass. With a sterile loop a large loopful of the liquefied agar medium is transferred to the sterile cover, and spread in a uniform, thin and circumscribed layer. This film of agar will solidify almost instantly and it is at once inoculated with the amœba culture. The cover-glass culture is then inverted over the concave centre of a hollow slide, which has been previously flamed, ringed with vaseline, and kept in an inverted position near at hand. This transfer of the cover-

glass culture to the slide can be accomplished either by picking up the cover with forceps and inverting it over the concave centre of the slide, or by pressing the inverted slide upon the cover glass, which adheres to the vaseline ring around the concavity of the slide. The cover is then pressed down around the edges until the vaseline completely seals the culture so as to prevent evaporation of moisture. Ordinarily the moisture of condensation from the solidifying agar and that transferred in inoculating the culture is sufficient for growth, but sterile distilled water or any media or reagents can be added with a sterile loop, either at the time of making the culture or later by raising the cover. On these cover-glass cultures amœbæ multiply as freely as on Petri plate cultures. The film of agar medium is thin enough to permit the use of a one-twelfth-inch oil immersion objective, and is so nearly transparent that it does not seriously obstruct the passage of light."

According to Walker the following are the essential factors in the cultivation of amœbæ on artificial media: (1) the consistence should be solid; (2) reaction should be neutral or preferably slightly alkaline; (3) bacteria should be present on which the amœbæ can feed; (4) media should be moist and oxygen should be present; (5) a temperature of 20 to 25° C. should be maintained.

For a full discussion of the methods used in the past and at present for the cultivation of amœbæ the reader is referred to the work of Musgrave and Clegg and to that of Walker.

I desire here to protest against the growing tendency of drawing conclusions regarding the morphology and the life cycle of the parasitic amœbæ as observed in man from organisms growing upon artificial culture media. Even if it were definitely proven that any of the parasitic amœbæ have been cultivated, deductions based upon the appearance of the amœbæ in such cultures would probably be erroneous, as it is well known that the cultural forms of protozoa so far described differ markedly in their morphology and life history from the forms observed in the human host. When we add to this fact the doubt which exists as to the cultivation of the parasitic species it is obvious that any attempt to describe or to liken the morphology of cultural forms to the parasites observed in man must result in failure and confusion. The entire subject of the cultivation of the parasitic amœbæ is in a chaotic condition and much more work will have to be done before it can be accepted that any of the parasitic species of man have been cultivated.

Major Whitmore of the U. S. Army Medical Corps, who has recently worked with Hartmann.

states in a letter to the author, that all of the cultures of amœbæ he obtained in Manila from dysenteric patients both from the feces and from liver abscess pus, as well as cultures from water, were found, in Hartmann's laboratory, to be free-living amœbæ and of no etiological significance in dysentery. This is in accordance with what I have always believed and stated, *i.e.,* that the parasitic amœbæ of man have not been cultivated.

VI.

THE AMŒBÆ OF THE INTESTINAL TRACT.

THE following amœbæ have been described as parasitic within the intestinal tract of man:

Genus, *Entamœba*.

Entamœba coli, Loesch, 1875, emend Schaudinn, 1903.
Entamœba histolytica, Schaudinn, 1903.
Entamœba tetragena, Viereck, 1907.
Entamœba minuta, Elmassian, 1909.
Entamœba nipponica, Koidzumi, 1909.
Entamœba tropicalis, Lesage, 1908.
Entamœba phagocytoides, Gauducheau, 1908.
Entamœba undulans, Castellani, 1905.

Genus, *Paramœba*.

Paramœba hominis, Craig, 1906.

ENTAMŒBA COLI. Loesch, 1875. Emend Schaudinn, 1903.

Before the publication of Schaudinn's researches observers had noticed the occurrence of amœbæ in the intestinal discharges of healthy individuals and those suffering from diseases other than dysentery, and this gave rise to the opinion that these organisms were

harmless and only of accidental occurrence in patients suffering from dysentery. However, as evidence of the pathogenic action of amœbæ accumulated it was found that such an opinion was untenable. In order to explain the pathogenic action of these organisms the theory regarding the effect of environment arose, together with the belief that more than one species of amœbæ occurred in the human intestine.

OCCURRENCE IN HEALTHY INDIVIDUALS.—It is surprising how little work has been done regarding the occurrence of amœbæ in the healthy human intestine. Although several years have elapsed since Schaudinn's paper was published, only a very few observers have undertaken the determination of how large a percentage of healthy individuals show amœbæ in the feces, and even those who have combated Schaudinn's classification have not taken the trouble to examine a large number of individuals in health, in order to prove or disprove his results.

Historical Summary.—Grassi, in 1888, was probably the first investigator to demonstrate the presence of amœbæ in the feces of healthy individuals, although Cunningham in 1881 stated that he found amœbæ in the stools in both healthy and diseased individuals, but his description indicates that he mistook stages in the life cycle of the flagellates for amœbæ. Shuberg demonstrated amœbæ in the feces of 10 out of

20 healthy individuals, or 50 per cent., while Gassard found 20 per cent. of infections in healthy individuals in examining 20 cases. Strong and Musgrave found amœbæ in only 4 per cent. of healthy individuals, but felt justified in believing that these amœbæ were not pathogenic because of the negative results of animal experiments. Dock examined 200 healthy people and only found amœbæ in 2 cases. He concludes his contribution to this subject as follows: "Even if a certain parasite occurs in every case in one locality, it would not follow that the same parasite would also be found as widespread elsewhere." Schaudinn, in 1903, published the results obtained by him in the examination of the feces of healthy individuals. He found that in West Prussia 50 per cent. of healthy men among the farming population showed amœbæ in their feces, while in Berlin only 20 per cent. were found infected; along the shores of the Adriatic Sea he found that in 385 examinations of as many individuals in perfect health, no less than 256 or 66 per cent. showed the presence of amœbæ. His observations, as well as those of others, demonstrate that locality and occupation have much to do with the number of individuals infected. In all the cases of healthy individuals examined by him the amœbæ found was *Entamœba coli.*

In 1905, I published the results obtained in

the examination of healthy American soldiers stationed at San Francisco, California. The examinations were made largely among the members of the Hospital Corps of the U. S. Army, recruited from almost every portion of the United States, and who were on duty at the General Hospital, and under constant observation. Over 200 men were examined and it was found that, after the administration of magnesia sulphate in ounce doses, the feces of 65 per cent. showed the presence of *Entamœba coli,* although in many instances repeated examinations were necessary in order to demonstrate the parasite. It is the writer's belief that the small percentage of positive results in the demonstration of this parasite, reported by some observers, are due to the examination of only one or two preparations. At least eight specimens should be examined before one can be sure that *Entamœba coli* is absent.

Believing that geographic distribution might have something to do with the proportion of infected individuals, the locality from which each individual came was ascertained, but there was apparently no great variation due to this cause. However, this negative result cannot be considered as conclusive and the question can only be settled when we have data concerning the examination of a large number of individuals in different localities. All the men examined

were in robust health, had never suffered from diarrhœa or dysentery, and presented no symptoms of either of these diseases. In some the amœbæ occurred in great numbers while in others a long search was required before they were demonstrated.

My results were confirmed, in 1906, by Captain Vedder of the Army Medical Corps, who examined the feces of 50 healthy American soldiers and 50 Filipino Scouts, the examinations being made in the Philippine Islands; of the American soldiers 50 per cent. showed *Entamœba coli* in their feces, while 75 per cent. of the Filipino Scouts were found to be infected with this parasite. Of the subsequent history of the cases examined by him, Vedder says: "All the men have been under observation for a period of nine months, and none of them has developed dysentery."

Major Ashburn and I, while serving in Manila upon the "Army Board for the Study of Tropical Diseases," examined 107 healthy American soldiers, all of whom were members of the Hospital Corps of the Army and all on active duty at the U. S. Army Division Hospital, Manila, P. I. Of the 107 men, 76 of 71+ per cent. were found to be infected with *Entamœba coli,* while two showed the pathogenic *Entamœba histolytica* in their stools. None of these men, with the exception of the two showing the patho-

genic amœba, had diarrhœa or dysentery at the time of examination, and all denied ever having suffered from dysentery symptoms since residing in the Philippines. Of seventy-two men showing *Entamœba coli* in their feces, one had resided in the Philippine Islands for eight years; four, seven years; one, six and a half years; three, six years; four, five and a half years; one, five and one quarter years; two, five years; four, four years; three, three years; two, two and a half years; ten, two years; one, one year and ten months; two, one year and nine months; nine, one and a half years; thirteen, one year; and the remainder, or seventeen, less than one year.

The two men showing *Entamœba histolytica* in their stools were apparently in good health, but inquiry elicited the information that both were suffering from dysenteric symptoms at the time of examination, and both were later returned to the United States suffering from chronic amœbic dysentery. At the time that we examined the feces of these men we knew nothing of the occurrence of the dysenteric symptoms, and our diagnosis was based entirely upon the morphology of the amœbæ observed in their feces. It will thus be seen, that contrary to the opinion of certain investigators, it is possible to differentiate *Entamœba coli* from *Entamœba histolytica* as they occur in the feces, and therefore, that such differen-

tiation becomes of very great importance in the diagnosis of diarrhœal conditions of the intestine.

In order to determine how long infection with *Entamœba coli* might exist we made the following examinations:

A. Upon November 20, 1906, thirteen men were re-examined who had been first examined upon March 17, 1906, eight months having elapsed since the first examination. Of these thirteen men, eleven showed *Entamœba coli* in their feces March 17, and nine or 81.8 per cent. still showed them upon November 20. During this time not one of these men had suffered from diarrhœa, and all had been on duty continuously at the hospital.

B. Upon November 20, 1906, seven men were re-examined who were first examined upon May 2, 1906, six months and twenty-two days having elapsed since the first examination. Of these seven men, five were positive for *Entamœba coli* upon May 2, and five were still positive upon November 20, and none of these men had suffered from diarrhœa or dysentery during this time, and were continuously under observation.

C. Upon November 20, 1906, eight men were re-examined who were first examined upon July 10, 1906, four months and thirteen days having elapsed since the first examination. Of these eight men, five

were positive for *Entamœba coli* upon July 10, and two were still positive upon November 20. Neither of these men had developed symptoms of diarrhœa or dysentery during this time.

As the result of our work we concluded that a large proportion of healthy white men serving in the Philippines harbored *Entamœba coli,* which infection, so far as we were able to observe, does not result in diarrhœa or dysentery, some of the cases being observed for 9 months without any symptoms developing.

Summing up the observations of the writer in San Francisco, in 1905, and those made in conjunction with Ashburn in Manila, we have the record of 307 examinations of as many healthy American soldiers, of whom 176 or 58+ per cent. showed the presence of *Entamœba coli* in their feces. Some of these cases have been followed for a period of three years and in none of them have symptoms of dysentery occurred. I have observed instances in which *Entamœba coli* has been constantly present in the feces for periods of from 4 to 6 years, and not the slightest symptoms of dysentery have developed.

In attempting to disprove the existence of *Entamœba coli* Musgrave has instanced cases in which amœbæ were found in the feces during apparent health, but in which dysentery followed at periods

varying from 2 to 6 months, and other cases in which death had occurred from other diseases and at autopsy the intestine was found to present the usual lesions of amœbic dysentery, although the patient had never complained of symptoms of that disease. As regards the first class of cases it may be said they are of no scientific value as proof that the amœbæ present in the beginning were pathogenic, for the patients were continually exposed to infection with *Entamœba histolytica,* in the intensely infected Philippine Islands; as regards the latter class of cases it is notorious that the Filipino frequently suffers from diarrhœa, although he generally does not consider this symptom of enough importance to mention when questioned, and most of the instances reported have been in Filipinos. After a long and extensive experience, acquired at the autopsy table, it is my opinion that it is impossible for *extensive* ulcerative lesions to occur in the intestine without producing symptoms of dysentery which would attract the attention of the patient. In cases where the lesions are localized in a small area the patient might pass a small amount of mucus or blood without knowing it, but such cases possess no value as proof of the non-existence of a harmless amœba, as both harmless and pathogenic species might occur in such individuals. Until it can be demonstrated that the lesions are the

result of infection with *Entamœba coli,* we cannot accept them as proof of the pathogenic character of this species.

Since the observations of Schaudinn and myself, numerous investigations have confirmed the occurrence of amœbæ, answering to the description of *Entamœba coli,* in a large proportion of healthy individuals. I believe that a considerable proportion of healthy individuals in almost any locality will be found to harbor *Entamœba coli* if the examination is carefully made. Schaudinn found this species in 50 per cent. of healthy individuals examined in West Prussia; in 20 per cent. examined in Berlin, and in 66 per cent. of those examined along the shores of the Adriatic; Craig in 65 per cent. of healthy American soldiers at San Francisco; Vedder in 50 per cent. of healthy American soldiers and in 72 per cent. of Filipino Scouts in Mindanao, P. I.; and Ashburn and Craig in 71 per cent. of healthy American soldiers in Manila, P. I. Our observations have since been confirmed by Major Whitmore, who found this parasite present in the Philippines, as well as by Hartmann. The percentage varies in different localities, but it is evident that *Entamœba coli* is a common parasite of man and that it does not produce the symptoms or lesions of amœbic dysentery.

Occurrence of Entamœba Coli in Diseases other than Dysentery.—As would be expected, this species of amœbæ not only occurs in health, but also in many patients suffering from diseases other than dysentery. Any condition which leads to the production of acute or chronic diarrhœa, such as acute or chronic enteritis, typhoid fever, or cholera, is often accompanied by the appearance of this parasite in the feces. Even in conditions in which lesions are not present in the intestine this parasite is frequently observed in the feces following the administration of a cathartic. Cunningham found amœbæ in the intestinal contents of cholera cases; Celli and Fiocca in infants suffering from intestinal inflammation; Berndt in cases of typhoid fever; Normand in chronic colitis; Massiutin in the feces of 5 cases suffering with intestinal catarrh, typhoid fever, and diarrhœa; Grassi in diarrhœa; Perroncito in chronic diarrhœa; Babes in cases of hepatitis; Casagrandi and Barbagallo in various diseases, as well as Kruse and Pasquale, Quincke and Roos, Kartulis, Shuberg, Gros, and Ijima.

In my own observations I have not confined myself to the examination of feces of patients suffering from diarrhœa, but have examined the excreta in all cases, whatever the diagnosis. Over 250 such cases have been examined of which 49 per cent. showed

the presence of *Entamœba coli*. It is significant that a smaller percentage of cases of disease show this amœba than of healthy individuals, which would appear to indicate that the parasite finds a more congenial environment for its development in the intestine of normal individuals. In all the cases in which diarrhœa was not present a saline cathartic was given before the examination, and in the patients in whom diarrhœa was present the occurrence of dysentery was excluded by the fact that they were under observation for weeks or months, during which time no dysenteric symptoms developed.

Table I gives the results obtained in the examination of 30 cases of various diagnosis, and is representative of the class of cases examined.

The occurrence of such a large number of instances of infection with *Entamœba coli* in patients suffering from diseases other than dysentery is of the greatest importance, especially in regions where amœbic dysentery is endemic. The examination of the feces of patients coming from such regions will, in a considerable proportion of the cases, result in the finding of this parasite, and an inexperienced observer would probably regard it as *Entamœba histolytica*, and a diagnosis of amœbic dysentery would be made. It is my belief that many practitioners have made this mistake and that some

of the marvellous cures attributed to certain remedies can be accounted for in this way. I have repeatedly observed cases diagnosed as amœbic dysentery upon the demonstration of *Entamœba coli* in the stools,

TABLE I.

EXAMINATION OF PATIENTS FOR ENTAMŒBA COLI.*

Number.	Diagnosis.	Residence.	Dysentery.	Diarrhœa.	In hospital.	Bowel movements.	*E. Coli.*
1	Anemia, secondary	England	No	No	70 days	2 daily	Present
2	Abscess of leg	Illinois	No	No	5 months	2 daily	Present
3	Abscess of axilla	....	No	No	20 days	2 daily	Present
4	Diabetes insipidus	Georgia	No	No	40 days	1 daily	Present
5	Fracture	Alabama	No	No	40 days	2 daily	Present
6	Gastritis, chronic	....	No	No	2 months	2 daily	Present
7	Gastritis, chronic	....	No	No	2 weeks	1 daily	Present
8	Gastritis, acute	California	No	No	1 week	1 daily	Present
9	Gonorrhœa, acute	Tennessee	No	No	1 month	1 daily	Present
10	Gonorrhœa, acute	California	No	No	1 month	1 daily	Present
11	Gonorrhœa, acute	Jamaica	No	No	10 days	2 daily	Present
12	Gonorrhœa, acute	Penna.	No	No	20 days	1 daily	Present
13	Gonorrhœa, chronic	Kentucky	No	No	35 days	2 daily	Present
14	Gonorrhœa, chronic	....	No	No	1 month	1 daily	Present
15	Gonorrhœa, chronic	Penna.	No	No	1 month	1 daily	Present
16	Hemiplegia	California	No	No	10 days	1 daily	Present
17	Pharyngitis, acute	....	No	No	10 days	1 daily	Present
18	Malarial Fever	Conn.	No	No	2 weeks	1 daily	Present
19	Malarial Fever	California	No	No	2 weeks	2 daily	Present
20	Malarial Fever	Virginia	No	No	2 weeks	1 daily	Present
21	Malarial Fever	....	No	No	2 weeks	1 daily	Present
22	Otitis Media	Texas	No	No	3 months	1 daily	Present
23	Measles	Texas	No	No	40 days	1 daily	Present
24	Measles	Texas	No	No	4 months	2 daily	Present
25	Pemphigus	Arkansas	No	No	5 months	1 daily	Present
26	Poliomyelitis	Miss.	No	No	4 months	2 daily	Present
27	Mitral Regurgitation	Penna.	No	No	10 days	2 daily	Present
28	Stricture	California	No	No	2 weeks	1 daily	Present
29	Sciatica, chronic	Missouri	No	No	3 months	1 daily	Present
30	Varicocele	Ohio	No	No	3 months	2 daily	Present

* This table includes only cases in which *E. coli* was found, in order to illustrate the variety of conditions in which this parasite may be present.

after a dose of salts, in which neither the clinical history nor the symptoms corroborated the diagnosis. A considerable proportion of the cases returned from the Philippine Islands during 1900 and 1901, and probably since then, diagnosed as amœbic dysentery,

were really cases of enteritis showing *Entamœba coli* in the feces. Aside from the zoölogical standpoint, it is of great practical importance that we recognize the fact that this non-pathogenic species occurs both in health and disease in the intestine of man and that we be able to differentiate between *Entamœba coli* and the pathogenic entamœbæ. This is often a very difficult matter when the organisms are few in number, and at certain stages of development, while it is always true that considerable experience in the examination of the feces is required before such a differentiation can be easily made. The exceedingly superficial study that many practitioners make of these parasites can never result in success in the differentiation of species.

Morphology in Fresh and Stained Preparations.—To secure living specimens of *Entamœba coli* it is always best to administer an ounce of magnesium sulphate or a Seidlitz powder before examining the feces, as this washes off the amœbæ from the mucous membrane and greatly facilitates their demonstration. The technique employed in preparing such specimens for examination has already been considered.

Entamœba coli consists of a mass of cytoplasm containing a well-defined nucleus, and in a few instances, one or more vacuoles. The differentiation between the ectoplasm and the endoplasm is not well

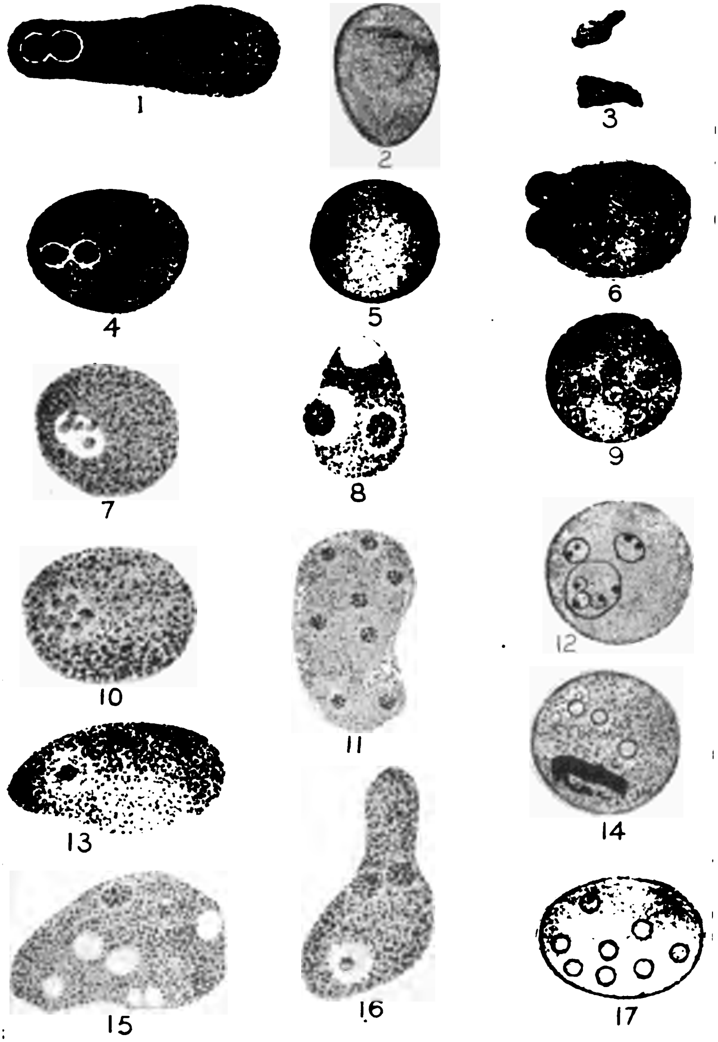

FIG. II.—*Entamœba coli.* (After Casagrandi and Barbagallo.) 15, 13, 10, and 7, various stages in nuclear division of *Entamœba coli;* 4 and 1, division of the nucleus into two as shown in the vegetative stage of growth; 16 and 11, schizogony and the formation of eight daughter-nuclei during the vegetative stage of development; 8, expulsion of a portion of the nucleus prior to encystment; 5, first stage of encystment showing the homogeneous cytoplasm; 2, oval encysted form with homogeneous cytoplasm; 17, encysted form within which eight daughter-nuclei have developed; 14, 12, and 9, illustrating various stages of nuclear division in the encysted forms; 6, two young amœbæ leaving the cyst in which they have developed; 3, two young amœbæ just after leaving the parent cyst.

marked, although the cytoplasm is thus divided. Motility is present, but is very sluggish as compared to *Entamœba histolytica.* Under favorable conditions reproduction occurs by simple division and schizogony, with the production of eight daughter amœbæ. If unfavorable conditions arise the organisms encyst and eight daughter cells are formed within the cyst, which are liberated under favorable conditions. The morphology of the parasite will be considered in detail, but it should be remembered that individual organisms vary greatly in appearance at different stages of development and that the description given is a composite picture which is true of the vast majority of parasites belonging to this species.

Size.—As a rule, *Entamœba coli* is not as large as *Entamœba histolytica,* although individuals are often observed which approximate the size of the latter organism. The measurements given by different authorities vary greatly, as one would expect, for during the different stages of development the parasites may measure from 5 microns to 50 microns in diameter, and as every specimen of feces will contain organisms in different developmental stages the variations in size reported do not indicate inaccuracy on the part of the observer. Schaudinn states that *Entamœba coli* varies between 8 and 50 microns in

diameter, but in my experience it is very rarely that one of these organisms exceeds 25 to 30 microns in diameter. It may be stated, as a general rule, that this parasite measures from 10 to 20 microns in diameter, while the average measurement of *Entamœba histolytica* is from 25 to 35 microns.

The difference in size between the two species is of but slight value in differentiation and a separation of the amœbæ of the human intestine into species, based upon the size of the organism, is both unscientific and erroneous. It is one of the least valuable of our methods of differentiation.

Some authorities have stated that pathogenic amœbæ are always larger than *Entamœba coli,* but I have very frequently observed cases of pure infection with *Entamœba histolytica* in which the amœbæ were as small as the ordinary *Entamœba coli.* As a matter of fact, both large and small amœbæ are present in every infection, so that it is impossible to differentiate between them on account of their size.

During the encysted stage of development *Entamœba coli* is much smaller than during its vegetative stage, the average diameter being from 10 to 15 microns.

Shape.—The shape varies with the motility of the organism. When motionless *Entamœba coli* is spherical or slightly oval in shape, but when in motion the

shape continually changes, depending upon the contour of the pseudopodia, but the latter are always rounded, and never spinose, as are the pseudopodia of many of the amœbæ cultivated from external sources. The writer has never observed an amœba in the feces having spinose pseudopodia, although free-living forms must frequently occur in the feces, especially in tropical countries, but probably in the encysted stage.

The shape of the pseudopodia is useful in differentiating this species from the pathogenic amœbæ, such as *Entamœba histolytica* and *Entamœba tetragena*. In the latter parasites the pseudopodia are generally finger-like in shape and of considerable size, while in *Entamœba coli* they are short and blunt. Aside from this characteristic of the pseudopodia, the shape of the organisms is of no value in the differentiation of species.

Color.—*Entamœba coli* may be said to be of a peculiar dull grayish color, and this is of some value in the differentiation of the species, the pathogenic amœbæ of the intestine appearing slightly greenish in color or almost colorless. Musgrave and Clegg believe that color simply means environment, the greenish color being due to the absorption of blood serum containing hæmoglobin, and that in feces in which blood is not present the amœbæ never present this

color. While this may be the reason for the greenish coloration frequently observed in *Entamœba histolytica,* it certainly does not apply in the case of *Entamœba coli,* for the addition of blood to the feces containing this organism does not result in the absorption of either dissolved hæmoglobin or the phagocytosis of red blood corpuscles.

It is extremely difficult to describe the exact color of this parasite, but to one who has studied the various species of amœbæ the difference in the coloring of *Entamœba coli* and the pathogenic amœbæ is quite characteristic.

Cytoplasm.—The cytoplasm of *Entamœba coli* is divided into an ectoplasm and an endoplasm. These two portions of the cytoplasm are exceedingly difficult to distinguish in all stages of the growth of the parasite, and impossible unless the organism is in motion, and in many specimens the ectoplasm cannot be distinguished from the endoplasm even when the parasite is actively motile. Schaudinn first called attention to this point and to the fact that the ectoplasm of *Entamœba coli* is much less refractive to light than is the endoplasm, in those instances in which the two portions can be distinguished.

The *ectoplasm* presents a homogeneous appearance and unless a high power lens is used no definite structure can be distinguished; with an immersion lens

it appears to be composed of very minute granules. When the parasite encysts, the ectoplasm is replaced by a refractive, smooth, or slightly wrinkled cyst wall, impervious to staining solutions and apparently of very dense structure. In some instances definite layers of a very refractive material compose the wall of the cyst, but generally it has a distinct double outline.

The *endoplasm,* constituting the greater portion of the cytoplasm, is of a finely granular structure and an examination with a high power lens shows it to be composed of a delicate network enclosing a fluid medium containing multitudes of fine granules. In most amœbæ numerous bacteria are observed in the endoplasm, as well as crystals derived from the feces, and in some organisms one, or perhaps two, small vacuoles. During reproduction by schizogony the endoplasm contains from 2 to 8 oval, slightly refractive bodies, which may be mistaken for vacuoles, but which are really the daughter amœbæ. The morphology of the parasite during this stage of development has led to the erroneous statement that *Entamœba coli* contains numerous vacuoles.

During the encysted stage the endoplasm is homogeneous in appearance and contains from 2 to 8 round, refractive bodies, representing the daughter amœbæ.

The entire cytoplasm in the vast majority of *Entamœba coli* appears to be composed of endoplasm, as the differentiation of the ectoplasm is impossible in most instances. In the very young amœbæ of this species the cytoplasm is homogeneous throughout.

During motion the pseudopodia, which are formed by the ectoplasm, appear to be somewhat less refractive than the endoplasm, and of very slight consistence. Sometimes the pseudopodia are only visible upon careful focussing, resembling a veil-like membrane projecting from some portion of the periphery of the parasite.

Vacuoles and Contained Bodies.—The vast majority of parasites belonging to this species present a finely granular cytoplasm in which no vacuoles can be observed. In the thousands of specimens studied by myself a vacuole was present in but a little over 10 per cent. of the parasites and a very small percentage showed more than one vacuole. When present the vacuole is of small size, and never contractile. The absence of vacuoles in this species is in striking contrast to the numerous vacuoles observed in *Entamœba histolytica,* and other pathogenic amœbæ, and is of some value in the differentiation of this species.

The endoplasm contains refractive dots and rods, some of which are micrococci, bacilli, and various crystals derived from the feces, while the nature of

others is still undetermined. These bodies are better differentiated in stained preparations.

Red blood corpuscles are rarely observed in the cytoplasm of *Entamœba coli,* and this point is of some value in the differentiation of the species. When present, the cytoplasm never contains more than one or two, and I believe that they are of purely accidental occurrence and that normally this parasite is not phagocytic for red blood corpuscles. Experimentally it is almost impossible to make these parasites engulf the erythrocytes when blood is added to feces containing them, although in the case of *Entamœba histolytica,* the engulfing of these cells occurs very frequently.

The development of the daughter-nuclei during schizogony has already been noted. These bodies appear within the cytoplasm as oval, slightly refractive areas, measuring from 3 to 5 microns in diameter and lying within the endoplasm. They may be easily mistaken for vacuoles by one untrained in the study of amœbæ. In stained specimens these bodies take the chromatin stain.

Nucleus.—The most prominent body in the cytoplasm of *Entamœba coli* is the nucleus, which is almost always distinctly visible, thus differentiating this parasite from *Entamœba histolytica,* in which the nucleus is generally invisible.

If one desires to study the changes occurring in the nucleus during reproduction the best results are obtained with special staining methods, and by the use of a warm-stage or an incubator in which the microscope can be placed during the examination. It is possible to observe division of the nucleus in the living parasite by the use of the warm-stage or incubator, but patience is required in order to demonstrate the changes which occur.

The size of the nucleus varies, but it generally measures from 5 to 8 microns in diameter. During certain stages of division the nucleus may be either larger or smaller than the average given, and during this period it may be hard to distinguish. In most instances the shape of the nucleus is spherical, but sometimes it is distinctly oval in contour. It is bounded by a well defined, heavy, nuclear membrane which in the living organism appears highly refractile. Upon the inner surface of this membrane there occur brightly refractile elevations, generally hemispherical in shape, consisting of nuclear chromatin, and dots and irregular granules of the same substance may be observed scattered throughout the nucleus. At or near the centre of the nucleus, during the vegetative stage of existence, there are generally observed from one to two very distinct masses of chromatin forming the karyosome. The substance of the nucleus, aside

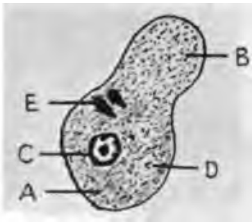

FIG. III.—Diagram of *Entamœba coli*. (Craig.) *A*, endoplasm; *B*, ectoplasm; *C*, nucleus, showing strong nuclear membrane and centriole; *D*, vacuoles; *E*, crystals absorbed from the feces.

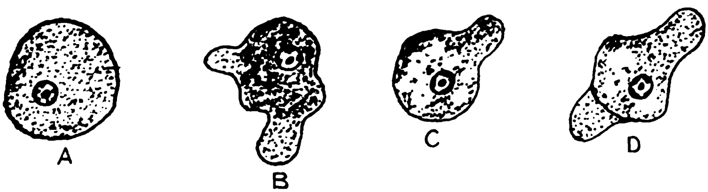

FIG. IV.—Changes in the form of *Entamœba coli* during amœboid motion. (Craig.) Note the lack of differentiation of the ectoplasm and endoplasm and the distinct nucleus.

from the chromatin, is apparently composed of fluid material enclosed in a very delicate achromatic network.

When the organisms are in motion the nucleus tends to retain its position near the centre, although at times it may be observed at the periphery of the moving parasite.

In some instances the limiting membrane of the nucleus is very thick and perfectly smooth in outline, while the chromatin is collected in irregular masses within the membrane, the karyosome being indistinguishable. This appearance is especially noticeable just prior to encystment and does not occur during the vegetative stage of existence. Again, amœbæ are observed in which the chromatin is arranged in very irregular masses upon the inner surface of the nuclear membrane or close to it, while the karyosome is well differentiated. Many different appearances as regards the situation of the chromatin are presented by the nucleus in various stages of development, but the most common are those which have been described.

Stained Preparations.—The methods which may be used for staining *Entamœba coli* have already been described, but I have found that Wright's method and iron hæmatoxylin give the best results. I have observed but little difference in the results obtained

with either wet or dry-fixed preparations, when Wright's stain is used, but if iron hæmatoxylin be used the wet-fixed preparations give the best results as regards the structure of the nucleus. Some authorities have claimed that unless the preparations are wet-fixed no deductions can be drawn regarding the structure of the nucleus in stained preparations, but with this statement I must entirely disagree. It makes but little difference whether the specimens be fixed wet or dry, when Wright's stain or any other modification of the Romanowsky stain is used, although the best results are obtained in wet-fixed preparations. I cannot but believe that many of the appearances presented in wet-fixed preparations are just as artificial as any that may be observed in the dry-fixed preparations, and there is considerable reason for concluding that both wet- and dry-fixed preparations should be used in the study of this class of protozoa.

When Wright's stain is used *Entamœba coli* presents three distinct portions, the ectoplasm, the endoplasm and the nucleus. However, it should be remembered that these three divisions are not always visible, as not infrequently the entire amœba stains uniformly throughout, with the exception of the nucleus. The period of development apparently makes a great difference in the facility with which the organism takes the stain, as well as with the reaction

to the stain employed, and in every preparation it will be found that the majority of the parasites stain but poorly, and that, as a rule, a large number of smears will have to be stained and examined before a good preparation will be found. If one attempts to follow the life cycle of the parasite in stained preparations it will be necessary to examine scores of smears before each step can be demonstrated, but if one has the patience it is possible to follow almost every stage of development in such preparations.

In those organisms in which the ectoplasm and the endoplasm are differentiated it is observed that the ectoplasm stains a very delicate blue and appears almost structureless, while the endoplasm stains a dark blue or violet, and is composed of deep blue granules and irregular masses of homogeneous material. The distinction between the light blue ectoplasm and deep blue or violet endoplasm is very marked and serves to differentiate this organism from *Entamœba histolytica,* in which the ectoplasm takes a deep blue, and the endoplasm a light blue color.

If one examines the well-stained organisms with a high power lens the ectoplasm will seem to be composed of very minute, dust-like, dimly stained granules, while the endoplasm contains deeply stained bacteria of various kinds and sometimes a few crystals. If vacuoles are present they are observed as un-

stained spherical or oval areas within the endoplasm.

With the Wright stain the nuclear chromatin takes a bright red or crimson color and the nuclear membrane stains very deeply, the chromatin situated upon its inner surface appearing as bright red or crimson masses. When over-stained the entire nucleus is bright red and no morphological details can be recognized. Within the substance of the nucleus the chromatin may be observed as delicate threads, rods, or granules, stained a bright red in color and separated by unstained spaces. The karyosome stains a deep red or violet and is usually well differentiated. Just prior to division, during the vegetative stage, the nuclear chromatin may be observed as two crimson stained masses lying at the poles of the elongated nucleus, but with this stain I have never observed distinct evidences of mitosis. In specimens stained with the iron-hæmatoxylin method evidence of a primitive mitosis are sometimes observed. The extrusion of the chromatin from the nucleus during certain stages of reproduction, first described by Schaudinn, is often evidenced in stained preparation by the presence of minute deeply stained granules of chromatin lying free in the endoplasm. The appearance of the nucleus in stained preparations during the various reproductive stages will be found described in the section dealing with this subject.

Motility.—Entamœba coli may be described as a sluggishly motile amœba in which this property is often absent, and when present, is of slight duration and very limited in extent. Motility is made possible by the extrusion of pseudopodia composed of ectoplasm, the character of which has already been described, but it may be recalled that they are small and rounded in contour and less refractile to light than is the endoplasm, while in many instances it is impossible to distinguish the boundary between the pseudopodium and the endoplasm, even when the organism is moving. Motility is always most marked in freshly voided feces and is seldom observed in feces which has stood at room temperature for more than one hour.

Two forms of motility are frequently observed; the first consisting in the extrusion of pseudopodia into which flows the endoplasm, thus producing a very sluggish progressive motion; the second, consisting in the extrusion of pseudopodia from different portions of the periphery at the same time, thus causing a change in the shape of the organism, but no progressive motion.

The sluggish motility of this species of amœba when compared with the active motility of such species as *Entamœba histolytica* and *Entamœba tetragena* is of considerable value in differentiation, for one never observes in *Entamœba coli* a progressive motion

which can be compared in activity with that commonly observed in the two other species mentioned.

Reproduction.—Before Schaudinn's researches little was known regarding the exact methods of reproduction of the amœbæ occurring in the human intestine. A few observers had roughly described certain reproductive phenomena, but as they were unable to distinguish species their descriptions are confused and of little value in the study of this subject. Celli and Fiocca, working in all probability with *Entamœba coli,* described the life cycle as consisting of an amœboid stage, a resting stage and an encysted stage, but their descriptions are incomplete, and it was not until the work of Casagrandi and Barbagallo appeared that the exact method of reproduction in *Entamœba coli* was known. They described simple division and the formation, under certain conditions, of cysts in which eight young amœbæ developed. Their work was confirmed and extended by Schaudinn, who found that reproduction during the vegetative stage occurs by simple division and by schizogony, with the formation of eight daughter amœbæ. When conditions are unfavorable for vegetative existence cysts are formed in which eight young amœbæ are developed. Complicated nuclear changes occur during schizogony and development within the cysts, which are best studied in preparations kept

FIG. V.— Photomicrograph of *Entamœba coli* and *Entamœba histolytica*. (After Jürgens.) The amœba at 1 is *Entamœba coli*, while the other amœbæ in the photograph are all examples of *Entamœba histolytica*. Note the well-defined nucleus in *Entamœba coli* and the absence of a distinct nucleus in *Entamœba histolytica*. Note the clear ectoplasm in *Entamœba histolytica*.

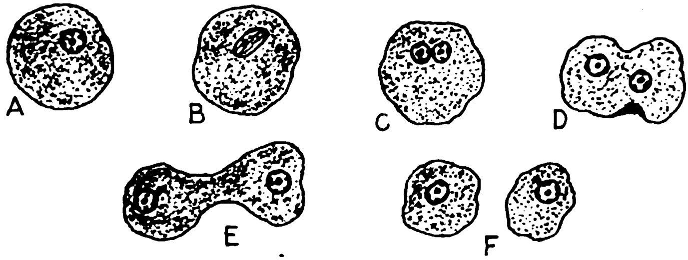

FIG. VI.— Multiplication by simple division in *Entamœba coli*. (Craig.) *A*, division of the chromatin within the nucleus into several clumps arranged upon the inside of the nuclear membrane; *B*, formation of a nuclear spindle, showing that the division of the nucleus is mitotic in character; *C* and *D*, division of the nucleus into two portions; *E*, division of the nucleus and partial division of the cytoplasm of the amœba; *F*, complete division of the parasite and the production of two amœbæ.

upon a warm-stage or under the incubator-microscope or in wet-fixed preparations stained with iron hæmatoxylin. I have been able to follow almost every developmental stage as described by Schaudinn and the following description of reproduction in *Entamœba coli* is based upon that given by Schaudinn and upon my own observations.

In reproduction by *simple division,* which is not infrequently observed in the feces, the following phenomena may be demonstrated in the living organism: the nucleus elongates, the nuclear membrane becoming thinner and less refractile while the chromatin, which is visible as brightly refractile dots or granules, becomes concentrated at each pole of the nucleus. After the nucleus has become much elongated a constriction develops near the centre and finally division occurs, two nuclei being produced. Coincident with the elongation and division of the nucleus the protoplasm of the amœba becomes less granular and a constriction appears which deepens and finally becomes complete after the division of the nucleus. In this way two amœbæ are formed and in stained specimens the division of the chromatin and the nuclear changes can be followed, the chromatin staining a bright red with Wright's stain. If iron hæmatoxylin be used, after wet-fixation, clear evidences of mitosis are frequently observed.

In reproduction by schizogony complicated nuclear changes occur resulting in the formation of eight daughter cells. The beginning of the process is first evidenced by swelling of the nucleus apparently due to the absorption of fluids from the endoplasm, while at the same time the latter extrudes all foreign material, and the entire organism becomes motionless. The chromatin situated upon the inner surface of the nuclear membrane divides into eight little heaps which finally become free within the nucleus, and this is followed by the rupture of the nuclear membrane and the liberation of the eight daughter nuclei within the endoplasm. After this occurs the cytoplasm of the amœba divides into eight more or less irregular portions each containing one of the daughter nuclei and in this way eight young amœbæ are produced. This process was first studied by Casagrandi and Barbagallo and the plate reproduced from their work well illustrates the daughter nuclei immediately after liberation from the nucleus.

In the living specimen the daughter nuclei are visible as brightly refractile masses of granules due to the chromatin of which they are largely composed, while in stained specimens the chromatin takes a bright red color when Wright's method is employed, so that this method of reproduction can be easily traced in such preparations.

Reproduction within a cyst occurs when conditions become unfavorable to vegetative existence. In freshly voided specimens of feces this process is very rarely observed, but in specimens which have been kept for some time this method of reproduction may be easily studied. If the infection has existed for some time encysted forms are more frequently observed in the freshly voided feces, for, like many other protozoan organisms, these parasites multiply for a long period of time asexually, but after a certain number of generations the process of reproduction assumes a sexual character, self-fertilization occurring within a cyst.

The cysts of *Entamœba coli* are spherical or oval bodies measuring from 10 to 15 microns in diameter, as a rule. The appearance of the cyst wall varies in different stages of development, but it is always refractile and generally presents a double outline. A mammillated cyst wall is sometimes noted, but is by no means commonly observed.

Just before encystment the amœba becomes perfectly motionless, while the endoplasm clears itself of granular material and all foreign particles. This process reduces the size of the organism about one-third, and after it is complete there forms upon the surface a very refractile, delicate, hyaline membrane which appears to be formed from the substance of

the ectoplasm. At first this membrane appears single in outline, but as reproduction proceeds a double outline develops and in some instances a well defined mammillation of the outer surface of the cyst wall occurs.

The cytoplasm, after the formation of the cyst wall, is perfectly hyaline in appearance and contains a nucleus in which the chromatin appears to have greatly increased in amount. If the cysts are placed under conditions favorable to development reproductive changes occur in the nucleus which may be briefly described as follows:

The nucleus first divides by a primitive mitosis into two daughter nuclei of the same size. These two nuclei move to opposite sides of the cyst and the cytoplasm gathers around them, dividing the organism into two partly separated portions. In the living specimen the nuclei are observed to disappear after several hours, but if stained specimens be studied it will be noted that the chromatin of the nuclei is very largely distributed to the cytoplasm, although a definite portion of the nucleus remains, and from this a new nucleus is formed. If living specimens be watched it will be observed that after the disappearance of the nuclei they will again reappear, but contain much less chromatin and are of smaller size.

Schaudinn describes certain variations in the primary changes occurring in the nucleus as follows:

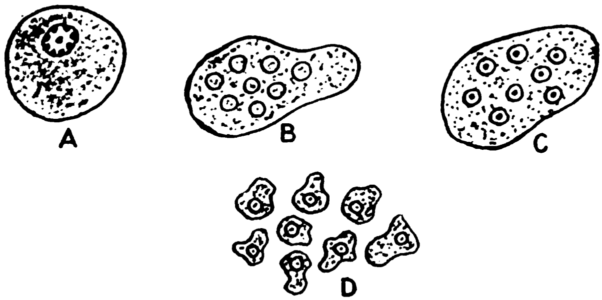

FIG. VII.—*Schizogony* of *Entamœba coli.* (Craig.) *A*, division of the nuclear chromatin into eight distinct masses arranged upon the inside of the nuclear membrane; *B*, division of the nucleus into eight daughter-nuclei; *C*, further stage of the same process of division; *D*, complete division of the amœba into eight daughter amœbæ.

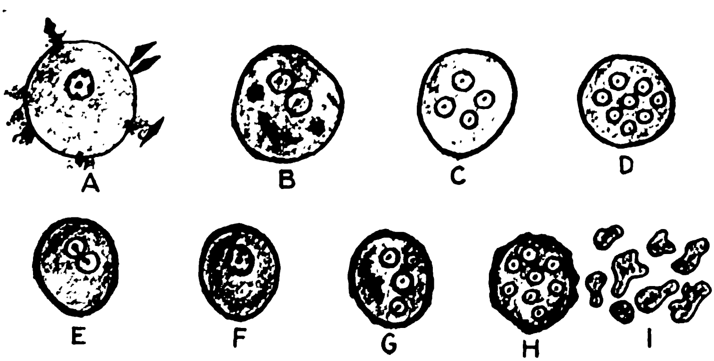

FIG. VIII.—*Sporogony* of *Entamœba coli.* (Craig.) *A*, an amœba ridding itself of extraneous matter just prior to encystment; *B*, division of the nucleus into two daughter-nuclei; *C*, division of the daughter-nuclei into two nuclei; *D*, eight nuclear stage of the cyst of *Entamœba coli; E*, *F*, and *G*, various stages in the sporogony of *Entamœba coli; H*, fully developed cyst of *Entamœba coli*, containing eight daughter-nuclei; *I*, the young amœbæ as they appear after liberation from the parent cyst.

1. The daughter nuclei may dissolve entirely, the plasma being filled with chromatin granules and cords, most of which disappear, while out of one part the new nuclei are reconstructed.

2. The daughter nuclei dissolve with the exception of a small central portion, while the chromatic granules and cords are partly expelled and partly resorbed.

3. The daughter nuclei extrude chromatic granules and cords to the plasma, but remain distinctly visible and are finally expelled from the cyst. In this case the new nuclei are formed from the free chromatin particles.

Other variations are noted, but are not of sufficient interest to be described. Schaudinn believes that the manner of reconstruction of the nucleus depends upon the degree of development of the cyst wall. He says:

"My observations showed to me that the indicated variability is connected with the degree of development of the cystic sheath. For if the reconstruction of the nuclei ensues while the gelatin sheath is only very weakly developed, or not at all, the casting out of the perishing parts of the nucleus is possible; but if the gelatin sheath is strongly developed or the differentiation of the real cystic membrane has begun, then the perishing substances remain in the plasma

and a complete dissolution of the nuclei takes place."

After the reconstruction of the daughter nuclei they are situated at opposite poles of the cyst and the following changes may then be observed:

Both divide by primitive mitosis so that two nuclei lie at the opposite poles. One of these contracts, forming a reduction body, while the other again divides. This results in the presence of three nuclei at each pole, two of them normal in appearance, while one is degenerating. One of the newly formed nuclei also degenerates eventually, leaving two nuclei at each pole of the cyst.

After this has occurred, the body of the amœba contracts still further and the cystic membrane becomes more pronounced, appearing firmer and more refractile. The division between the two portions of cytoplasm is lost, the two pair of nuclei divide mitotically, two daughter nuclei being formed, called the active and passive pronuclei. These pronuclei fuse, forming two synkarya, each of which divides into four, so that the cyst now contains eight daughter nuclei. During this time the walls of the cyst have become firmer and thicker and there is no indication of division of the cytoplasm. Under favorable conditions, such as reception into a new host, the cyst wall dissolves, the cytoplasm divides into eight irregular masses each containing a daughter nucleus, and in

this way eight young amœbæ are produced. Schaudinn states that of the cysts voided with the feces only from 10 to 20 per cent. undergo reproduction in the manner described, and this agrees with my own observations. Many preparations have to be studied before the entire process of reproduction can be followed, but sometimes one is so fortunate as to be able to follow the entire development in a single individual.

The process of reproduction within a cyst has to be studied from living preparations entirely, as I have not found any method of staining the cysts nor have I been successful with any method recommended by other observers.

CULTIVATION.—I believe that it may be stated with truth that *Entamœba coli* has never been artificially cultivated. I have used all the methods which have been recommended for this purpose, but without success.

RELATION TO DISEASE.—The wide distribution of this parasite in both healthy individuals and those suffering from diseases other than dysentery; the fact that scores of cases have been observed in which the parasites have been constantly present for months and even years without producing symptoms of diarrhœa or dysentery; and the negative results obtained by animal experiments, all definitely prove that *Entamœba coli* is not a pathogenic species. It may be of

interest to review briefly the experimental work relating to the pathogenicity of *Entamœba coli.* Kartulis experimented with amœbæ obtained from healthy individuals by injecting them into the intestine of cats, but was not able to produce any pathological lesions or symptoms of disease. The same negative results were obtained by Kruse and Pasquale. One of the strongest arguments used by Celli and Fiocca against the pathogenic action of amœbæ was the fact that they were not able to produce dysentery in cats by the injection of amœbæ from healthy individuals, and the very careful experiments of Kovacs were equally unsuccessful. Strong and Musgrave were also unable to produce dysentery in cats with the amœbæ found in health, and their comment regarding their negative results is of interest. They say " one of these cases which has been under our observation for several months has had these harmless amœbæ in his stools constantly during that time, yet he has no dysentery and no history of any, and he has no intestinal trouble. We have repeatedly injected large numbers of these non-dysenteric amœbæ (amœba coli) while motile in the stools, into the rectum of cats, but with no effect. We have neither been able to produce dysentery with them nor any lesions of the large bowel—on the other hand we have had no difficulty in producing dysentery and

ulcerations of the large bowel in cats by the injection of the stools or contents of liver abscesses containing motile amœbæ dysenteriæ."

The work of Jürgens is of special interest in connection with *Entamœba coli*. He demonstrated that this parasite is not able to penetrate the normal mucous membrane of the intestine because of the slight strength of the pseudopodia and his observations have been confirmed by Schaudinn. The latter observer was unable to produce dysentery or any lesion of the bowel in animals by feeding experiments or the rectal injection of material containing *Entamœba coli*. He was successful in infecting young cats and many of his studies upon reproduction were made on amœbæ obtained from such animals, but the infection never resulted in either symptoms or lesions of dysentery. He twice infected himself by swallowing encysted forms, in both instances the experiment being controlled by regular examinations of his feces for two months before the experiment. After swallowing the cysts numerous amœbæ appeared in his feces, but in neither instance did any symptoms of diarrhœa or dysentery develop and the amœbæ gradually disappeared.

My personal observations cover a large number of experiments in which young kittens were used as the animals experimented upon. I have injected into

the rectum of kittens fecal material containing both encysted and motile forms of *Entamœba coli* and have never been able to produce the least symptom of diarrhœa or dysentery, although 50 per cent. of kittens injected with material containing *Entamœba histolytica* developed dysentery, of which many of them died. I have repeated these injections upon the same animal from 5 to 10 times and in no case was any evidence of intestinal inflammation produced.

I have made many feeding experiments in which kittens were given milk containing multitudes of encysted and motile forms of *Entamœba coli* and in not a single instance were any symptoms of diarrhœa or dysentery produced, although the feedings were repeated at frequent intervals. In kittens fed with milk containing *Entamœba histolytica,* on the other hand, over 65 per cent. developed severe dysentery.

To one who has carefully followed the growth of our knowledge concerning the amœbæ of man it is impossible to doubt that *Entamœba coli* is a harmless commensal occurring in a very large percentage of healthy individuals and in individuals suffering from diseases in which inflammatory conditions of the intestine can be excluded. Were this parasite the cause of a form of dysentery, as is still believed by some, practically 50 per cent. of individuals in nearly every locality would suffer from this disease. All of the

arguments which have been brought forward in the endeavor to show that this species is simply a non-virulent form of the amœbæ commonly found in dysentery, have one by one been abandoned in the face of the evidence which has accumulated as to the specific nature of the parasite.

The recognition of this species is of practical importance because many patients have been diagnosed as suffering from amœbic dysentery upon the mere presence of *Entamœba coli* in the feces. I have known patients to be treated with rectal injections for weeks in whom the only evidence of dysentery was the presence of amœbæ in the stools, which upon careful examination proved to be *Entamœba coli*. It is undoubtedly true that the statistics of the Army regarding the occurrence of this disease in the Philippines are greatly vitiated by records of diagnoses based upon the mere presence of amœbæ in the feces, no effort having been made to differentiate the species. It must be admitted that such differentiation cannot be made by the tyro in this work, but trained laboratory assistance is generally available in most localities and a correct diagnosis is of enough importance to warrant submitting material to an expert. It is just as reasonable to advocate treating every case of fever with quinine on the supposition that the malarial plasmodia are present, as it is to treat all

individuals showing amœbæ in the stools for amœbic dysentery. This advice has been given by some authorities, but if it were followed out, over one-half of the population of many tropical localities would be under treatment for amœbic dysentery. This is well illustrated by the observations of Ashburn and myself in Manila, P. I., where we found over 72 per cent. of the Hospital Corps men on duty at the Division hospital infected with *Entamœba coli*. It is not difficult to imagine the condition in this hospital had we advocated placing these men under treatment, and yet this is just what is recommended by those who refuse to accept Schaudinn's classification. The fact that it is difficult in many instances to differentiate the non-pathogenic species from the pathogenic is no excuse for failure to do so, for so much depends upon the diagnosis of amœbic dysentery that it should never be made unless the pathogenic species has been demonstrated in the feces.

The reports made by a few observers, notably Musgrave, of severe lesions of dysentery being found at autopsy, although no symptoms of the disease had ever been present, must be doubted. I have autopsied hundreds of cases of amœbic dysentery and I have never yet observed a case in which well marked lesions were present but that a history of attacks of diarrhœa or dysentery could be obtained. It is im-

possible to believe that marked lesions of this disease can occur in an intestine without leading to the production of clinical symptoms. It is possible that in isolated instances a few ulcers might exist in limited regions of the intestine without producing symptoms which would attract the attention of the patient, but that any generalized ulceration can occur without symptoms I am not ready to believe. The lack of a history of diarrhœa or dysentery cannot be depended upon in native races, for such symptoms are not considered of enough importance by these people to be noted. Almost all of Musgrave's cases were in Filipinos who suffer continually from diarrhœa and who would not be apt to notice a symptom which is more or less constantly present. We have no record of such cases occurring in temperate regions among people in whom the symptoms would be apt to attract attention.

However, the occurrence of the lesions of amœbic dysentery in patients who have not suffered from symptoms of that disease is of no value as an argument against the specificity of *Entamœba coli,* and I believe there is sufficient evidence at hand to prove conclusively that this species is valid and that it occurs commonly as a harmless commensal in the human intestine.

ENTAMŒBA HISTOLYTICA. Schaudinn, 1903.

This species was first differentiated by Schaudinn in 1903, although it had been previously studied in a thorough manner by Kartulis, Jürgens, Councilman and Lafleur, Strong and Musgrave, and many others. It is a pathogenic species, causing amœbic dysentery, its distribution probably being world-wide, although it occurs most frequently in tropical and sub-tropical regions. The pathological lesions produced by it, as well as the experimental evidence, prove conclusively that it is the cause of a distinct form of dysentery which is endemic in many localities and which may become epidemic when conditions are favorable.

Geographical Distribution.—This species of amœbæ has been demonstrated in the Philippine Islands, by Ashburn and myself, Vedder, and other observers; in Formosa, by Nakagawa; in Cochin China, by Pfuhl and other French investigators; in Siam, by Wooley; in India, by Fearnside, Powell, Rogers, Viereck and Duncan and Anderson; in Africa, by Kartulis, Marchoux, Ruge, A. Plehn, Wellman, and Prout; in Europe, especially in Austria and Poland, by Hlava and many other observers; in South America, by Dessy and Marotta; and in the United States, by Osler, Musser, Dock, Ellis, Tuttle, Boggs, Stockton, Patterson and others.

In a collective study of the occurrence of amœbic dysentery in the United States, Patterson records cases reported from Maine, New Hampshire, New York, Pennsylvania, Maryland, District of Columbia, Virginia, West Virginia, North Carolina, South Carolina, Georgia, Florida, Tennessee, Alabama, Mississippi, Ohio, Illinois, Missouri, Michigan, Minnesota, Montana, Arkansas, Indian Territory and Texas. Long has reported numerous cases originating in California and it is very probable that in this State the infection was imported by soldiers returning from the Philippine Islands.

While the disease is wide-spread in the United States and is probably much more common than is generally supposed, it has nowhere been reported in epidemic form, but occurs as isolated cases in many of which the exact origin of the infection is problematical. *Entamœba histolytica* is generally considered as the exciting cause in the infections reported as originating in this country, but unfortunately the species present have not been studied thoroughly and it may be that many of these cases are really due to a distinct species endemic in the United States. I have recently found *Entamœba tetragena* in cases originating in the United States as well as *Entamœba histolytica.*

MORPHOLOGY.—Like *Entamœba coli* this parasite

consists of a mass of cytoplasm containing a nucleus and varying in shape when in motion, although it is always spherical or oval in outline when motionless. The cytoplasm contains a nucleus and one or more vacuoles which are not contractile. The cytoplasm is divided into two distinct portions, the ectoplasm and the endoplasm, the ectoplasm being clear and glass-like in appearance, while the endoplasm is less refractile and more granular in structure. The endoplasm contains the nucleus, vacuoles, and may contain red blood corpuscles, bacteria, crystals, or other material which may have been engulfed by the parasite. The nucleus is generally invisible and contains a very minute karyosome. Movement is accomplished by the extension and retraction of very distinct pseudopodia formed by the ectoplasm. Reproduction occurs by simple division and by sporulation or gemmation.

The following detailed description of the morphology of this parasite is the result of careful study of many thousand of these organisms and refers only to the morphology of the amœbæ as they are observed in the feces. I have not been able to obtain cultural forms of this parasite, although some authorities have claimed to have done so.

Size.—The size of *Entamœba histolytica* is variously stated by different observers, but it may

IX

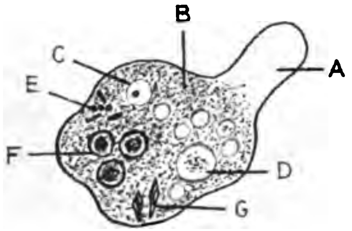

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FIG. IX.— Diagram of *Entamœba histolytica*. (Craig.) *A*, ectoplasm; *B*, endoplasm; *C*, nucleus, showing absence of a well-defined nuclear membrane, and the minute centriole; *D*, vacuole; *E*, bacteria; *F*, red blood-corpuscles; *G*, crystals.

FIG. X.— *Entamœba histolytica*. (After Hartmann.) Showing the character of the nucleus and karyosome. A red blood-corpuscle is present enclosed within a nutritive vacuole.

XII

XI

FIG. XI.—*Entamœba histolytica*. (After Viereck.)

FIG. XII.— Photomicrograph of *Entamœba histolytica*. Note the clear ectoplasm and the large number of red blood-corpuscles contained within the parasite.

be said that it generally measures from 25 to 50 microns in diameter. In the vast majority of instances this parasite is considerably larger than *Entamœba coli,* but, of course, the size varies with the stage of development. However, I am of the opinion that the size is generally under- rather than over-stated by most writers. From my own observations I believe that *Entamœba histolytica,* during its vegetative stage, is seldom less than 15 microns in diameter, and generally much larger. If we remember that the average blood corpuscle measures 7 microns in diameter it will be at once apparent that we seldom see amœbæ in cases of dysentery which are as small as one or two of these cells. It is also well known that this species is phagocytic for red blood corpuscles and it is not at all unusual to see from 3 to 6 of these cells within the small amœbæ. I have observed amœbæ of this species containing from 10 to 30 red blood corpuscles and this gives a definite idea of the large size of some of these parasites. A measurement of 50 microns in diameter is not infrequently observed and the vast majority of these parasites measure from 30 to 35 microns, a much greater average measurement than that of *Entamœba coli.*

These remarks do not apply to the encysted stage of development or to the young spores. The cysts measure from 10 to 20 microns in diameter, while the spores average about 5 microns in diameter.

The size of this species is of some value in differentiating it from other amœbæ, especially *Entamœba coli,* but it cannot be depended upon alone. Large and small amœbæ occur in almost every specimen of feces examined, so that a classification based upon size alone must be erroneous. Some investigators have endeavored to make the distinction into pathogenic and non-pathogenic amœbæ rest simply upon the size of the organism, the larger organisms being classed as pathogenic, and the smaller as non-pathogenic. From my experience I cannot agree with these conclusions. Careful examination of the feces of cases of amœbic dysentery often show that amœbæ of large size are not always present, while in other cases both large and small amœbæ belonging to this species are found. I am free to confess that as a rule the majority of the organisms are approximately of the same size in most specimens of feces, but this does not prove anything more than that they are nearly all in the same stage of development. I have repeatedly observed cases of dysentery in which the vast majority of the amœbæ present in the feces were much smaller than the average given for *Entamœba histolytica,* but in which the clinical symptoms were the same as in the cases where the larger amœbæ were found, while the morphology of the organisms was identical with that of *Entamœba histolytica.*

The size of this parasite is of importance in diagnosis as there is no parasite which has been so frequently mistaken for epithelial cells or for leucocytes when in the non-motile condition. When moving the organism is easily recognized, but when non-motile it is most difficult to differentiate from other cells occurring in the feces. In fact it has been stated by some good observers that it is not safe to diagnose the presence of amœbæ in feces unless motility is observed, but while this may be good advice for the beginner, it is too general a statement, for if the organisms are not undergoing degeneration they are easily recognized even when non-motile by one who has had experience in the study of this class of parasites.

If it is remembered that these organisms are larger, as a rule, than the leucocytes or intestinal epithelial cells; that they generally contain red blood cells; and are divided into two distinct portions, an ectoplasm and endoplasm; it should not be difficult to differentiate them from other bodies occurring in the feces.

Shape.—When resting, *Entamœba histolytica* is generally spherical in shape, although it is not unusual to observe oval organisms. When in motion great variation in shape is observed, due to the pseudopodia, so that it is impossible to accurately describe the contour of the parasite at this time.

Color.—I have already called attention to the dull grayish color of *Entamœba coli* and to the lack of differentiation between the ectoplasm and the endoplasm. In *Entamœba histolytica* the ectoplasm is almost colorless and very refractile, resembling a piece of ground white glass, while the endoplasm is of a very light gray color, in many instances tinged with green, due to absorbed hæmoglobin. In cases in which the stools contain blood, the majority of the amœbæ observed show this greenish tint due, I believe, to hæmoglobin liberated during the digestion of the red blood corpuscles. That this is true can be experimentally demonstrated if one cares to spend the time to do so. I have many times observed an amœba cross the microscopic field and engulf a red blood corpuscle and have watched the latter slowly disappear, the endoplasm at the same time assuming a greenish color.

It is very common to observe not only well preserved red blood cells within this organism, but also fragmented corpuscles, and in such instances it is always noted that the endoplasm is distinctly green in color. This phenomenon proves that *Entamœba histolytica* is capable of engulfing and destroying the red blood corpuscles of its host and serves to distinguish it from *Entamœba coli,* for in the few instances in which I have observed red blood corpuscles

within the latter organism, I have never seen any evidence indicating that the amœbæ were able to digest them. I believe that in *Entamœba coli* the engulfing of red corpsucles is purely accidental, and that the cells are extruded without being digested.

The Protoplasm.—The appearance of the protoplasm varies considerably with the age of the parasite. In the small or young amœbæ, the protoplasm is finely granular in appearance and the ectoplasm and endoplasm can seldom be distinguished unless the organism is in motion, but in the larger and older parasites this distinction can frequently be made even when motility is absent. The protoplasm contains a nucleus situated to one side of the centre of the parasite, which is generally invisible. One or more vacuoles are present, as well as crystals, bacteria, pigment granules and amorphous material.

The Cytoplasm.—In the fully developed parasite the cytoplasm is divided into two distinct portions, the ectoplasm and the endoplasm. The ectoplasm comprises about one-third of the cytoplasm and is perfectly hyaline and glass-like in appearance. If a high power lens is used it appears to be composed of dense material containing innumerable very minute granules. It is very refractile, much more so than the endoplasm, and is easily recognized. The ectoplasm gives one the impression of a firm structure

capable of penetrating soft tissues and Schaudinn claims that it is owing to this property that these organisms push their way into the intestinal wall. He says:

"The harmless *Entamœba coli* with its soft pseudopodia is unable to penetrate the healthy epithelial layer of the intestine, while the dysentery amœba by means of its tough ectoplasm can do so. This is easily observable upon fresh sections of infected cat intestines in which the amœbæ will crawl about for hours, forcing the cells of the epithelium asunder, thus working their way into the tissues."

One has but to compare the firm appearance and well defined ectoplasm of *Entamœba histolytica* with the delicate ectoplasm of *Entamœba coli* to be convinced of the truth of Schaudinn's and Jürgens's assertion that the secret of the pathogenic action of *Entamœba histolytica* lies partly in the ability of the ectoplasm to penetrate the mucous membrane of the intestine, a property not possessed by the ectoplasm of *Entamœba coli* because of its delicate structure.

The *endoplasm,* which comprises about two-thirds of the body of the amœba, is light grayish in color and is composed of granular material enclosed within an ill-defined reticular structure. It is much coarser than the ectoplasm and less refractile.

Inclosed within the endoplasm there are generally

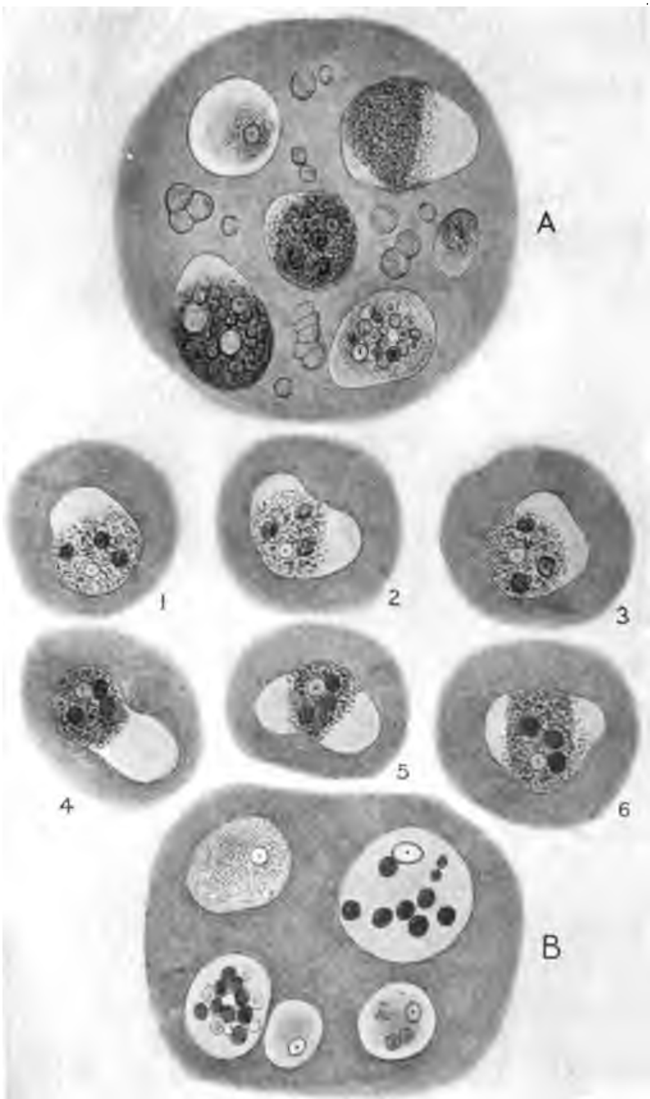

FIG. XIII.—*A*, living *Entamœba histolytica* from feces of dysentery, showing nucleus, differentiation between ecto- and endoplasm, vacuoles, and red blood-corpuscles; *B*, dead amœbæ, showing loss of distinction between ecto- and endoplasm and the more definite appearance of the nucleus; 1 to 6, changes occurring in the shape of *Entamœba histolytica* during amœboid motion for one and one-fourth minutes. Objective 2 mm. homog. Immers. compensating ocular, No. 4. (After Jürgens.)

one or more vacuoles, non-contractile in character. In some of the larger parasites these vacuoles are very numerous, and such organisms are probably degenerating. Besides the vacuoles, small oval bodies are often observed, the nature of which is still in doubt, although they are generally interpreted as being the nuclear portion of the spores which bud from the organism. Red blood corpuscles, crystals, and bacteria are generally observed lying within the endoplasm.

The Nucleus.—It is generally difficult to distinguish a nucleus in this species, a fact which serves to assist in differentiating it from *Entamœba coli,* the nucleus in the latter being visible in nearly every organism as a distinct body possessing a well marked nuclear membrane, masses of chromatin and a definite karyosome. The nucleus of *Entamœba histolytica* is generally situated excentrically in the endoplasm, often near the boundary of the ecto- and the endoplasm, or it may be in contact with the border of the ectoplasm, being flattened out against it. The size varies with the stage of growth of the parasite, but it averages about 5 microns in diameter. When the organism is moving the nucleus continually changes its position and it is exceedingly difficult to study it at this time, owing to its continual disappearing from view within the endoplasm.

A well defined nuclear membrane cannot be distinguished, the border of the nucleus being of the same refraction as the hyaloplasm. By very careful focussing it is sometimes possible to demonstrate a very delicate limiting membrane, slightly more refractile than the rest of the nucleus.

The amount of chromatin contained within the nucleus of *Entamœba histolytica* is much less than in the nucleus of *Entamœba coli*. A small chromatic zone is frequently present at the border of the nucleus and a few minute granules are sometimes observed within the hyaloplasm, but this is in marked contrast to the nucleus of *Entamœba coli*, in which the chromatin is distinctly visible, both as well defined elevations upon the inner surface of the thick nucleus membrane and as masses of granules within the hyaloplasm. The relative proportion of chromatin and its arrangement is well shown in the specimens stained by Wright's method, which will be described later.

A karyosome is situated near the centre of the nucleus and is very small and delicate in appearance.

The nucleus is most easily observed in young amœbæ and the reason for this is demonstrated in stained specimens where it is noted that in the larger amœbæ the chromatin of the nucleus is distributed to the cytoplasm, the remainder of the nucleus forming a residual body, irregular in shape, which it would

be very difficult, if not impossible, to demonstrate in the living specimen. During reproduction by gemmation the nucleus is seldom visible in the living preparation, but during simple division it can generally be distinguished, lying near the centre of the parasite.

The characteristic features of the nucleus of *Entamœba histolytica* are its lack of a well defined nuclear membrane, the small amount of nuclear chromatin, the minute karyosome, often invisible, and the fact that it is generally invisible or distinguished with great difficulty.

Vacuoles and Contained Bodies.—The endoplasm of this species generally contains one or more vacuoles which are not contractile. In the young parasites a vacuole may be absent, but generally a single one is present, while in the fully developed organisms from one to ten or even more may be present, the average number being two or three. If only one vacuole be present it is of large size and spherical in shape, but if they are multiple the size varies and some of them appear oval in outline.

In many instances the vacuoles appear to contain bacteria, hæmoglobin, small refractile granules, or other material, suggesting that they are digestive in character, but I have never observed any evidence that they are contractile. When the organisms are in motion the vacuoles are continually changing their position within the endoplasm.

In some amœbæ the vacuoles comprise nearly all of the substance of the organism, the ectoplasm being invisible, while the endoplasm consists merely of a network enclosing the numerous vacuoles. Such amœbæ are undoubtedly undergoing degeneration, for though they may retain their motility for some time, it will be observed they eventually undergo fragmentation. The significance of the vacuoles in *Entamœba histolytica* is still uncertain. The presence of various substances within them suggests that they have something to do with digestion, but the absence of contractility places them apart from the class of contractile vacuoles which are present in many free-living amœbæ. However, it is probable that they have a distinct function, although when they are present in large numbers I believe that they are due to degeneration of the cytoplasm.

One of the most characteristic biological features of *Entamœba histolytica* is its power of phagocyting the red blood corpuscles of its host. This property can be easily demonstrated by adding fresh blood to specimens of feces containing motile amœbæ, and there can be no doubt but that the red corpuscles are digested within the parasites. These cells are very commonly observed within this species, not only when the feces contain blood, but often in cases where there is no macroscopic evidence of its presence. The

number of red cells which may be contained within an amœba is sometimes enormous, and I have often observed organisms so filled with these cells that very little of the structure could be distinguished.

In cases of amœbic dysentery in which both *Entamœba coli* and *Entamœba histolytica* are present it is but seldom that the former contain red blood corpuscles while the majority of the latter may be filled with them. As a rule only two to six red cells are contained within an amœba, and if the process of digestion is to be studied an organism should be selected which contains from one to two red corpuscles. If such organisms are kept at body temperature the red cells will be seen to gradually break up, the hæmoglobin being liberated, imparting to the endoplasm a greenish color. Extrusion of the red cell is sometimes observed, but this is an abnormal process due to unfavorable environment, except in those organisms in which reproductive changes are occurring.

In many amœbæ the red corpuscles become decolorized, only a delicate shadow of the cell remaining visible. Such decolorized corpuscles are often mistaken for small vacuoles, but careful focussing will reveal their nature. These shadow-corpuscles must also be differentiated from the refractile oval bodies to be mentioned.

Besides the vacuoles and red corpuscles the endoplasm generally contains pigment granules, crystals of various kinds, and bacteria. In addition there occur small oval bodies giving the reaction of chromatin when stained, which form the chromatic portion of the nucleus of young amœbæ which are developing within the parent organism.

Motility.—In fresh specimens of feces *Entamœba histolytica* is actively motile, much more so than *Entamœba coli.* This property is rendered possible by the pseudopodia, which in this species are well differentiated, appearing clear, refractile, and firm in consistence. To study the production of the pseudopodia it is necessary to keep the specimen at body temperature, and if this is done motility is maintained for hours. Even in specimens of feces kept at room temperature the motility of this parasite is often retained for a long time, motile amœbæ being sometimes observed after from two to six hours. The rate of motility is of some value in differentiating this species from *Entamœba coli,* in which motility is always feeble. The mere extrusion of pseudopodia is more rapid, even though progressive motion may not occur, while the division between the ectoplasm and endoplasm is very distinct.

The shape of the pseudopodia is of importance in the diagnosis of this organism. It will be re-

XIV XV

FIG. XIV.—Photomicrograph of *Entamœba histolytica*. (After Jürgens.) Note the clear ectoplasm and the large number of red corpuscles within the amœbæ, also the absence of a visible nucleus. × 1000.

FIG. XV.—Photomicrograph of *Entamœba histolytica*. (After Jürgens.) Note the presence of a small, illy-defined nucleus, much extraneous material and the clear, well-defined ectoplasm. × 1000.

A B C

FIG. XVI.—*A*, *Entamœba histolytica*, showing nucleus and two red blood-corpuscles. (After Roemer.) *B*, *Entamœba histolytica* filled with red blood-corpuscles. (After Roemer.) *C*, *Entamœba histolytica*, containing eight red blood-corpuscles and showing a well-defined ectoplasm. (After Roemer.)

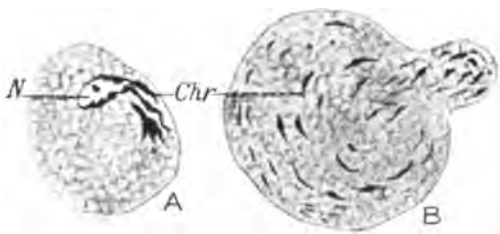

FIG. XVII.—*A*, *Entamœba histolytica*, stained, showing degeneration of the nucleus and the escape of chromatin into the cytoplasm. (After Hartmann.) *B*, *Entamœba histolytica*, stained, showing distribution of the chromatin throughout the cytoplasm and the disappearance of the nucleus. (After Hartmann.)

membered that in the *Entamœba coli* the pseudopodia are short and blunt, but in this species they are generally finger-like in shape and of large size.

Three forms of motility may be distinguished: active progressive motion, the extrusion of pseudopodia without progression, and movements of the cytoplasm.

The character of the progressive motion in freshly passed feces varies considerably, but it is generally rapid when compared to the sluggish motion of *Entamœba coli*. As the feces become cool progressive motion is gradually lost and, in such specimens, may be entirely absent or so sluggish as to require careful and prolonged observation to distinguish it. The organism advances by throwing out clear pseudopodia into which the endoplasm flows. The shape of the pseudopodia varies from a round, broad mass of ectoplasm to long slender processes with rounded extremities. Pointed pseudopodia so frequently observed in free-living species of amœbæ are never observed in any of the parasitic species of man. The long, slender pseudopodia are most frequently observed in the rapidly moving organisms, the more rounded pseudopodia occurring in amœbæ possessing sluggish motion.

The pseudopodia are always hyaline in appearance when first extruded, but the endoplasm quickly flows

into them, giving them a granular appearance, and in rare instances amœbæ of this species are observed in which the motion is so rapid that the distinction between ectoplasm and endoplasm cannot be made. In *Entamœba coli* it is often very difficult to distinguish these two portions of the cytoplasm even when the organism is in motion.

In *Entamœba histolytica* the flowing of the endoplasm into the pseudopodia generally occurs very rapidly and it often appears as though the periphery of the endoplasm ruptured, allowing the contents to rush through into the pseudopodia. Not infrequently a constriction is present in the pseudopodia near the boundary of the endoplasm, and when this is the case, the contents may be seen to pass slowly through the constriction, the nucleus, vacuoles and red corpuscles being compressed as they pass through the narrow portion.

When motility is pronounced the amœbæ generally progress in a definite direction or across a microscopic field without cessation of progressive motion; in other instances motion will occur for a short distance in one direction, followed quickly by progression in another, so that it may be a long time before the amœba will pass out of the field of a one-sixth-inch objective.

The second form of motility is frequently ob-

served in organisms which have been exposed to room temperature for some time, and consists in the active extrusion of pseudopodia unaccompanied by progressive motion, the processes of ectoplasm being continually projected from the periphery of the parasite and as quickly withdrawn. In such instances the endoplasm does not flow into the pseudopodia, as a rule, but occasionally this occurs, though progressive movement is prevented by the projection of new pseudopodia. This form of motility is only observed in organisms which have been exposed to unfavorable conditions, as lower temperature and solutions of chemical substances, so that it is fair to assume that it is an evidence of abnormal environment.

The third variety of motion is rarely observed. It may be called an intra-protoplasmic form of motility, consisting of currents evidently produced within the endoplasm. The contents of the endoplasm are observed to be in motion in a circular manner, the nucleus, vacuoles, red blood cells, and other substances being whirled about within a boundary formed by the ectoplasm, while an undulatory motion of the latter is sometimes observed. The movement of the current is generally slow, but it may be very rapid, and may continue for several minutes.

Of the significance of this form of motion we are ignorant. It is somewhat similar to that occurring

in *Balantidium coli* just prior to encystment, and also to the whirling motion of *Paramœba hominis,* a species of amœbæ parasitic in man, in which encystment is always preceded by this form of motility. However, in the case of *Entamœba histolytica* I have never observed any evidence of encystment, although such organisms have been watched for hours. In addition, progressive motion may be resumed after this form of motility has occurred, thus indicating that it has nothing to do with encystment.

STAINED PREPARATIONS.—This species of amœbæ may be stained with any of the methods which have been described, the preparations having been either wet- or dry-fixed. The best results are obtained with Wright's stain, using wet-fixed preparations or with iron hæmatoxylin preparations which have been fixed while wet. It is always difficult to stain these parasites and many preparations will have to be examined before one can expect to obtain material for a careful morphological study. I have found that Wright's stain gives good results and that with it one is able to follow the changes that occur in the nucleus during reproduction.

It is also possible with this stain to differentiate this species from *Entamœba coli,* the larger parasites being differentiated by the deep blue staining of the ectoplasm and the dim staining of the endoplasm, the

opposite being true of *Entamœba coli.* In the smaller amœbæ this distinction cannot be easily made, but every specimen of feces containing this species will show some organisms in which the distinctive staining of the ectoplasm and endoplasm will be found.

The nucleus of this species stains very poorly as compared with that of *Entamœba coli,* on account of the small amount of chromatin which it contains, as well as from the fact that in many of the amœbæ the nucleus is undergoing division prior to sporulation. With Wright's stain the nuclear chromatin stains a pale red or pink, while if iron hæmatoxylin be used for staining the nucleus is dark blue or almost black in color. As a rule, the nucleus appears larger in Wright- or Giemsa-stained preparations and the chromatin granules appear more irregular and massive.

METHODS OF REPRODUCTION.—We owe our first accurate description of the methods of reproduction of *Entamœba histolytica* to Schaudinn, and upon the difference in these methods from those of *Entamœba coli* he very largely based his classification of these two species. The difference in the methods of reproduction of *Entamœba histolytica* and *Entamœba coli,* amply suffices to establish the two species, even though we had no other evidence of their specific nature.

Entamœba histolytica reproduces by simple division by budding or gemmation, and by sporulation.

Simple division occurs as in *Entamœba coli,* the nucleus dividing into two almost equal portions followed by the division of the cytoplasm, which results in the production of two motile amœbæ. Schaudinn described the division of the nucleus as amitotic in character, but if the iron-hæmotoxylin method of staining be employed, the division is seen to be mitotic, the karyosome dividing with the formation of a nuclear spindle and a central spindle formed by the division of the centriola. The observations concerning the mitotic division of the nucleus in this species have been confirmed by H. Werner, who describes the process very accurately.

Reproduction by budding or *gemmation* consists in the formation of small daughter amœbæ which are pinched or budded off from the periphery of the mother organism. This process is initiated by the distribution of the nuclear chromatin to the cytoplasm, a portion of the nucleus undergoing degeneration. The chromatin collects into small meshes forming a portion of the nucleus of the young amœbæ, and is finally extruded from the parasite, together with a portion of the cytoplasm. This process is quite similar to sporulation, but does not result in the formation of resistant spores. In specimens stained with iron hæmatoxylin the chromatin appears in the form of dots and threads distributed in the cytoplasm

but with the Wright or Giemsa stain it appears in irregular masses.

Reproduction by sporulation only occurs after vegetative reproduction has taken place for many generations. Until the researches of Schaudinn this method of reproduction had not been observed in any amœbæ, but since his researches were published it has been confirmed by numerous investigators and serves to distinguish this species from the other intestinal amœbæ. In view of the interest attaching to Schaudinn's work I shall quote in full his description of this method of reproduction.

"As in many other parasitic protozoa the formation of these stages in *Entamœba histolytica* occurs only after a lengthy period of lively increase, when the conditions of life have deteriorated. In dysentery this is simultaneous with the commencement of healing. Permanent forms ensue when the feces become firmer—or in more correct language, healing begins when the vegetative increase of the amœbæ ceases. During the height of the disease I have never found these permanent stages.

"The beginning of spore formation is first noticed in the nuclear apparatus. The peripheral chromatin zone of the nucleus broadens and extends into the nuclear plasm, the nucleus at the same time becoming

less differentiated and surrendering large quantities of chromatin to the cytoplasm. In the stained specimens the casting off of these chromatin granules can be followed step by step. The amount of chromatin in the cytoplasm increases until the entire organism appears to be filled with it while the remainder of the nucleus degenerates.

"In observing these forms during life the following phenomena are noted: The nucleus is located at the periphery of the parasite generally in the shape of a flat disc at the border of the ectoplasm. Sometimes it is entirely expelled while under the eye of the observer. The peripheral ectoplasm portion appears at first entirely homogeneous, but as the process proceeds a fine fibrous structure is observed parallel to the surface, indicating the formation of the buds which finally project from the surface of the organism. Gradually these small elevations multiply, rise higher upon the surface, and finally separate in the shape of small globules measuring from 3 to 7 microns in diameter. In a short time these globules without changing in structure will develop upon their surface a colorless double outline membrane which in a few hours becomes brownish in color, the interior of the globule appearing structureless, while the parent-amœba gradually breaks up and disappears.

"A staining of this series of stages gives the

following results. The nucleus gives off chromidia to the cytoplasm, which appear to multiply and scatter through the entire organism, the nucleus degenerating and either entirely dissolving or being extruded. The chromidia withdraw from the endoplasm and collect in the dense fibrous tissue of the ectoplasm, finally permeating the latter as a uniform reticular chromidial mass. Ectoplasma buds filled with a chromidial mass then protrude upon the surface of the parasite, and are finally budded off from the parent body. As soon as the sheath of these globules is formed staining substances no longer act well.

"If the yellowish brown sheath now forms, even the stained preparations will no longer give information concerning the structure of the interior of the globules, and section technique likewise fails, for the small globules do not yield to the knife. Hence I am unable to state anything in regard to the nuclear changes occurring in the interior of these spores."

Prior to the observations of Schaudinn regarding this method of reproduction, I published a description of *Entamœba histolytica* in which I mentioned the occurrence of bodies within the cytoplasm which I thought might be spores. In concluding this paper I said:

"There occur in all but the degenerative forms of the amœbæ small round or oval, dimly stained areas, uniform in appearance, and most numerous in the large full-grown forms, being entirely absent in the vacuolated shells of amœbæ. These areas resemble similar areas in stained segmenting malarial plasmodia, and which are in them due to the young spores. Reasoning from analogy it may be that these areas in the amœbæ are also spores."

After the appearance of Schaudinn's work it was very apparent that the bodies which I described were in reality the small masses of chromatin which form a portion of the nucleus of the young spores.

I have been able to confirm Schaudinn's description of this method of reproduction and the following description gives the results of my studies of this process as observed in living and stained preparations of *Entamœba histolytica,* using both wet and dry fixed specimens.

It is not unusual to observe in the feces amœbæ in which the endoplasm contains refractile granules and rods collected in irregular masses or distributed throughout the organism. These bodies are the chromidia which have been liberated by the breaking up of the nucleus. Werner considers that part of the chromidia is derived from the peripheral nuclear chromatin and part of it is produced by the karyo-

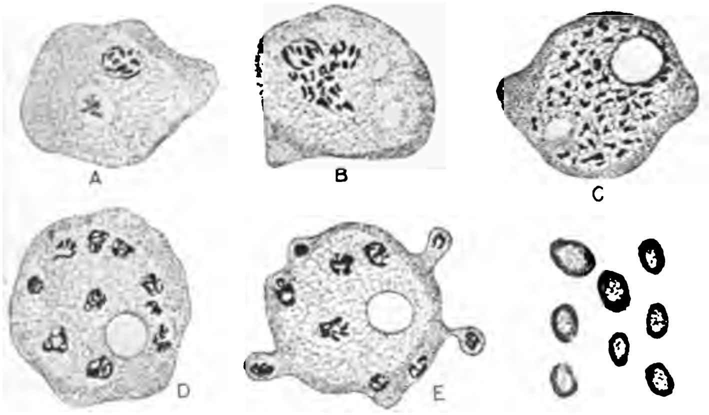

Fig. XVIII.—Reproduction by budding in *Entamœba histolytica*. (Craig.) *A*, organisms showing rods and granules of chromatin in the nucleus, a vacuole containing some stained substance and the deeply stained ectoplasm; *B*, illustrating the passing of the nuclear chromatin into the cytoplasm, where it becomes distributed as chromidia, shown in *C*; *D*, the formation of secondary nuclei by aggregations of chromidia; *E*, the formation of spores by budding; *F*, spores of *Entamœba histolytica* after liberation from the parent organism.

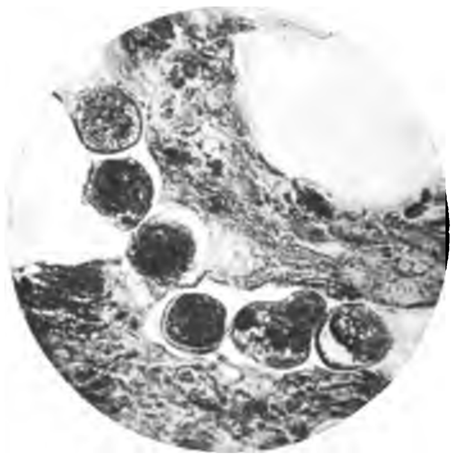

Fig. XIX.—A section of the intestine showing *Entamœba histolytica* within the mucous membrane. × 750.

some. The chromidia appear as brightly refractile granules and delicate threads with enlarged ends. Other organisms are observed in which the chromidia are collected in masses near or within the ectoplasm, sometimes distending it. This appearance is caused by the grouping of the chromidia prior to the liberation of the spores. Still other organisms are observed in which refractile masses of chromatin, surrounded by a small portion of cytoplasm, are in a process of separation from the parent organism.

The spores in the fresh specimens of feces appear as round or oval bodies having a yellowish refractile membrane and homogeneous contents. They measure from 3 to 7 microns in diameter and on account of their color they resemble red blood corpuscles, but may be differentiated from them because of the surrounding membrane.

If it is desired to follow the method of reproduction in the stained preparations a large number of specimens must be examined and Wright's stain or better, iron hæmatoxylin, used for this purpose. The larger amœbæ should be selected for study and the following forms illustrating various stages in the process will be observed:

1. Amœbæ in which the chromatin of the nucleus stains a light pink and is arranged in the form of delicate rods and granules.

2. Amœbæ in which the chromatin is situated partly within the nucleus and partly near it, in the endoplasm. The chromatin appears to have increased in amount and stains more intensely. This form illustrates the beginning of the distribution of the chromidia to the endoplasm and the degeneration of the nucleus.

3. Amœbæ in which the chromatin is distributed in very faintly stained grains and rods throughout the endoplasm, the remainder of the nucleus having disappeared.

4. Amœbæ in which the chromatin is collected into small clumps situated near or in the ectoplasm. These organisms represent the stage just before sporulation.

5. Amœbæ in which some of the masses of chromatin are arranged within the ectoplasm causing it to project slightly, and in which one or more of the clumps of chromatin, surrounded by a little protoplasm, is partially or entirely separated from the parent organism. In some of these an unstained area will be observed surrounding the spore, indicating the beginning of the formation of the sheath.

From the description of the various forms which may be observed in stained preparations it is evident that the entire process of reproduction described by Schaudinn can be followed and the observations of numerous investigators have confirmed his results.

This method of reproduction definitely differentiated this species of amœba from *Entamœba coli,* in which reproduction occurs in an entirely different manner. If Schaudinn's classification rested entirely upon the marked differences in the reproductive cycle of these two organisms it would be unassailable, but in addition we have marked differences in morphology, and the experimental evidence of the effect of the two species upon susceptible animals.

Conjugation.—I have several times observed a process which may be interpreted as conjugation in this species of amœba. Two organisms may sometimes be noted lying in contact, while marked streaming of the protoplasm of each is present. It often appears as though there was an interchange of protoplasm and I am sure that at times I have seen the nucleus of one within the cytoplasm of the other. Beside the streaming motion of the protoplasm the organisms are frequently observed to apparently revolve about one another, while still attached, this movement alternating with the motion of the protoplasm.

Conjugation has also been described in this species by Werner, and he states that one of the organisms appears to differ from the other, being clearer and more homogeneous in structure, but I have not been able to notice any difference in the appearance of the conjugants.

It is impossible at present to be sure of the exact nature of this process, but it appears to me more than probable that it is an instance of true conjugation. Such a process has been described as occurring in *Balantidium coli* and I have also observed it in *Entamœba tetragena.*

RELATION TO DISEASE.—At the present time almost all authorities are convinced that certain forms of dysentery are due to amœbæ, but there are still a few students of the subject who maintain that the amœbæ are only secondary invaders. In support of their position they claim that these organisms are often found in the feces of healthy individuals and of those suffering from other diseases; that direct infection with amœbæ has never been proved; that deductions based on experiments upon cats, monkeys, and other animals are unsatisfactory, because such animals suffer naturally from dysentery; that the presence of amœbæ in feces is a natural condition, such organisms being normal inhabitants of the intestines; and finally that the pathogenic amœbæ have never been cultivated.

These arguments can all be satisfactorily answered by the results of research accomplished during the past seven years. We now know that the presence of amœbæ in healthy individuals and in those suffering from diseases other than dysentery is explained

by the existence of a non-pathogenic species, *Entamœba coli;* that experiments upon animals *are* reliable provided they are properly controlled; that direct infection of susceptible animals has been abundantly proven; and that the argument that the pathogenic species have not been cultivated is not a valid one as disproving their relation to dysentery, as many other organisms undoubtedly connected with disease have not been cultivated.

In support of the causative relation of certain species of amœbæ to dysentery we have the following facts:

1. The absolutely characteristic pathology of amœbic dysentery.

2. The constant presence of the pathogenic species in the characteristic lesions and their absence from the lesions of other kinds of dysentery.

3. The constant presence of the amœbæ in the peculiar form of abscess of the liver complicating amœbic dysentery.

4. The experimental production by feeding and inoculation experiments, of a disease in susceptible animals presenting the same pathological lesions as those of human amœbic dysentery.

The evidence mentioned above applies in the case of *Entamœba histolytica* and *Entamœba tetragena,* the two pathogenic species which had been thoroughly

studied, and this evidence will now be considered in detail.

The Pathology of Amœbic Dysentery.—It is not my intention to discuss fully the pathology of the form of dysentery due to *Entamœba histolytica,* but to simply call attention to the characteristic lesions of this condition. It is now well recognized that great epidemics of dysentery occur in which amœbæ cannot be demonstrated in the lesions of the disease, and that such epidemics are due to a group of bacteria. This type of the disease is known as bacillary dysentery, and the lesions present differ markedly from those found in amœbic dysentery. In the bacillary type the characteristic lesion is a general superficial ulceration of the mucous membrane of the colon accompanied by intense congestion, while in the more chronic forms the ulcers may penetrate into the submucosa and a general gangrenous condition of the bowel may result, but to one who has had experience in autopsy work upon this form of dysentery a glance is sufficient to distinguish it from the amœbic type. The lesions of the latter form of dysentery cannot be confused with those found in any other variety, although some authorities have tried to prove that the lesions of amœbic dysentery do not differ markedly from those of the bacillary type. Such statements can only be based upon a very limited experience for

even the tyro will not be confused if he has seen a few cases of both varieties upon the autopsy table. I have performed several hundred autopsies upon cases of dysentery, including both the bacillary and amœbic types, and can state positively that it is impossible to confuse the pathology of the amœbic type with that of the bacillary.

The lesions of amœbic dysentery are most commonly observed in the rectum and just below the ileocæcal valve. In mild cases they may be confined to one of these regions, while in the most severe cases the entire colon may be invaded. As showing the relative frequency of the lesions in various portions of the large intestine I may say that out of 78 cases no less than 57 showed lesions below the ileocæcal valve and in the rectum, the intervening portion of the intestine being uninvolved; twelve cases showed lesions extending the entire length of the colon, but invariably most severe in the rectum and below the ileocæcal valve; while the remaining nine showed lesions only in the rectum and for a short distance above the sigmoid flexure. In only two of the cases that came to autopsy have I observed any extension of the disease above the ileocæcal valve.

The most characteristic lesions of the early stage of amœbic infection are small nodular areas which project from the summit of the folds of the mucous

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membrane into the lumen of the intestine. The mucous membrane covering them is generally inflamed, and when incised they are found to contain a yellowish or a greenish-yellow viscid fluid of a gelatinous consistence, which, upon microscopic examination, is seen to be composed of degenerated cellular material, mucus, and actively motile amœbæ. These nodular elevations mark the situation of the ulceration which develops later.

The next stage of amœbic dysentery is indicated by the appearance of small ulcers formed by the necrosis of the mucous membrane covering the nodular elevations just mentioned. The base of these ulcers is surrounded by inflamed mucous membrane, while the edges are ragged in appearance, and the floor, situated in the submucous coat of the intestine, is covered with the gelatinous material which has been described. The ulcers spread by invasion of the surrounding structure both laterally and downward, becoming larger and larger, finally reaching the muscular coat of the intestine. In some instances the small ulcers are pretty generally distributed and all of about the same size, but in a majority of cases both the small and large ulcers occur together, and in many cases one may trace the entire pathology of the disease, from the initial lesion, represented by the intact nodular mass, to the large ulcer involving all of the coats of the intestine.

The invasion of the mucous membrane laterally leads to the formation of sinuses beneath this membrane, connecting the neighboring ulcers. This lesion is one most characteristic of amœbic dysentery and does not occur in any other form. Upon opening such sinuses it is generally found that they contain gelatinous material similar to that contained within the nodules and microscopic examination reveals the presence of numerous amœbæ.

In aggravated cases the necrosis of the mucous and submucous coat of the intestine as well as the coalescence of smaller ulcers, leads to the formation of irregular ulcers of large extent, the mucous membrane between them presenting deep, irregular channels produced by the necrosis of the tissue covering the sinuses, while the whole surface of the intestine is covered with partly detached shreds of necrotic membrane. This so-called "buffalo skin" appearance is very frequently present in severe cases of amœbic dysentery and is very characteristic of that disease.

It is very difficult to describe the various forms of ulceration which may be present in the intestine in amœbic infection. A typical ulcer may be said to have the following structure: the edges are considerably raised from the surface of the mucous membrane and are much undermined, presenting a very characteristic shaggy appearance due to necrotic tissue; the

floor of the ulcer may be rough or smooth, the older ulcers having a smooth floor while in the more recent ones the floor is covered with necrotic material, pus, and blood. In the majority of the ulcers the floor is formed by the submucous coat of the intestine, but in all advanced cases ulcers will be observed in which the floor is formed by the muscular coat of the intestine. The smaller ulcers are generally round or oval in shape, but the larger ones are more irregular in shape. The ulcers vary in size from 0.5 cm. to from 8 to 10 cm. in diameter. Frequently the entire mucous membrane of a considerable portion of the intestine has been destroyed by the coalescence of two or more large ulcers, and I have repeatedly observed ulcers encircling the bowel and measuring as much as 6 to 8 cm. in the short diameter.

The mucous membrane between the ulcerations in mild infections may appear normal, but in the more severe types it is always inflamed, thickened, and covered with mucus and pus. The muscular coat of the intestine appears to offer a barrier to the extension of the disease, but this coat may be destroyed and perforation of the intestine may occur. In chronic cases the muscular and submucous coats of the intestine become greatly thickened and sometimes almost cartilaginous in consistence.

The exact manner in which the amœbæ produce

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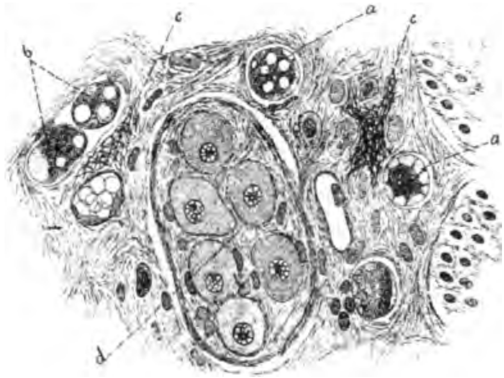

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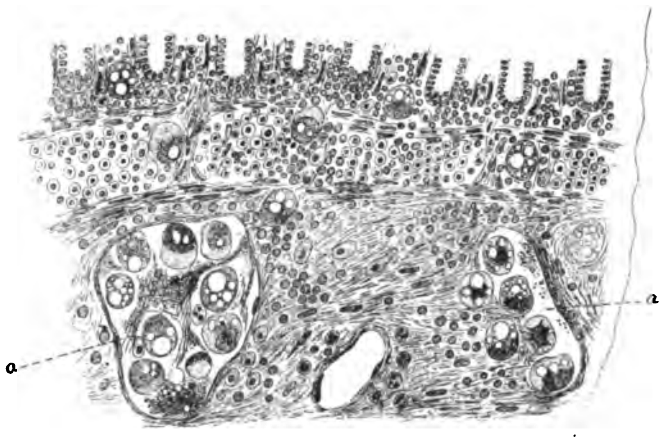


Figs. XX and XXI.—Sections of the intestine showing numerous amœbæ within the tissues. (After Councilman and Lafleur.)

lesions of the intestine is not known. That they are able to penetrate the epithelial lining of this organ has been abundantly proven by the work of Jürgens, Schaudinn, and others, and by their presence in the tissues as shown in microscopic sections. It is possible that they secrete a toxin which causes the initial destruction of the epithelial cells which enables them to penetrate beneath the mucous membrane, and that this toxin has much to do with the production of the lesions in the deeper tissues, but of this we have no definite proof.

In microscopic sections of the intestine of patients dying of amœbic dysentery, it may be stated that, almost without exception, the amœbæ are present in the necrotic tissue forming the wall of the ulcers and in the surrounding tissue. They are most abundant in the submucous coat, but are often observed in the muscular coat when the ulceration has extended to that region. When present they are always surrounded by numerous leucocytes and other evidence of inflammatory action. They are most numerous in the areas which are situated nearest the region of greatest degenerative changes, and it is rather rare to find them in the necrotic tissue overlying the ulcers or composing the edges. Amœbæ may be observed lying within the lymph spaces and sometimes within the lymphatics and veins. When the disease has ex-

tended to the muscular coat the amœbæ are found lying in the intermuscular septa and in this situation they may be observed arranged in rows and, where the tissue is not compact, scattered in irregular groups. In whatever region they are present, however, there is always evidence of an inflammatory process.

It is difficult to say how much of the pathologic process present is actually due to the amœbæ, for after the formation of ulcers we must remember that we are dealing with a mixed infection. Multitudes of bacteria are present which may have much to do with the morphology of the lesions, but the fact remains that these lesions are not the same in appearance as those produced by bacteria alone, thus indicating that the characteristic picture of amœbic infection is in all probability due to the specific action of the amœbæ.

There are three lesions present in this disease which are absolutely diagnostic:

1. The nodular thickenings situated in the mucous membrane which, when incised, are found to be filled with gelatinous material.

2. Ulcers having greatly thickened walls raised from the surrounding mucous membrane and presenting shaggy, yellowish-brown edges, which are always undermined. These ulcers are often covered by

necrotic membrane which upon removal reveals the interior of the ulcer filled with mucus, pus or blood, the floor being formed by the submucous or muscular coat. The presence in advanced cases of numerous ulcers causes the interior of the intestine to resemble the rough, yellowish-brown, shaggy appearance presented by old buffalo skins.

3. The almost invariable presence of irregular sinuses connecting the ulcers and situated beneath the mucous membrane.

Abscess of the Liver.—A considerable proportion of patients suffering from amœbic dysentery develop abscess of the liver, and it is now a well established fact that the pathogenic amœbæ cause this peculiar form of liver abscess.

The frequency of this complication is variously stated by different observers, according as the percentage is based upon autopsy reports or upon the total number of cases observed. Kartulis found that 55 per cent. of five hundred cases coming to autopsy showed amœbic abscess of the liver; Zancarol found that amœbic abscess of the liver occurred in 59 per cent. of 444 cases that came to autopsy; Smith in 45 autopsies upon amœbic dysentery cases found this complication in 84.4 per cent.

Councilman and Lafleur collected the data upon 1429 cases of amœbic dysentery, of which only 21

per cent. were complicated by abscess of the liver. In 745 cases of amœbic dysentery which I observed, in which amœbæ were demonstrated in the feces, abscesses occurred in only about 5 per cent., but in 78 fatal cases this condition was observed in nearly 33 per cent., and this well illustrates the difference in statistics obtained by considering the subject from these two standpoints. However, I must admit that the percentage is much lower in the cases I have observed than is usually reported.

The number of abscesses present in the liver varies from one to 20 or more. For many years it was considered that the single abscess was characteristic of the amœbic type, but this statement is not borne out by autopsy experience. While the single large abscess is often observed in these cases, multiple abscesses are almost as frequently observed, and in my experience over 50 per cent. of fatal cases have shown multiple abscesses. Sometimes the entire liver is filled with them, but in such instances there is always a mixed infection and many of the abscesses are due to bacteria.

The amœbic abscess occurs most frequently in the right lobe of the liver, the favorite location being at the dome, close to the attachment of the diaphragm, or the under surface near the hepatic flexure of the colon. Even in cases where multiple abscesses are present the largest and oldest abscesses are almost

invariably found in the right lobe. I have observed but three cases in which the findings at autopsy proved that abscess formation was confined to the left lobe. My experience in this respect has been borne out by others, especially by Rouis, who collected 639 cases of abscess of the liver in which the location of the abscesses was as follows: 435 or 17.8 per cent. were situated in the right lobe; 85 or 13.3 per cent. were situated in the left lobe, and 2 or 0.3 per cent. in the lobus Spigelii.

The following table illustrates the location of the abscesses in 24 cases which I have observed at autopsy:

TABLE II.

ILLUSTRATES THE NUMBER AND LOCATION OF ABSCESSES IN TWENTY-FOUR FATAL INFECTIONS WITH ENTAMŒBA HISTOLYTICA.

No. of case.	Abscesses.			
	Single.	Multiple.	Number.	Location.
1	...	Yes	6	Right lobe 5.............Left lobe 1
2	...	Yes	8	Right lobe 8.............Left lobe 0
3	...	Yes	4	Right lobe 3.............Left lobe 1
4	...	Yes	13	Right lobe 8.............Left lobe 5
5	...	Yes	8	Right lobe 8.............Left lobe 0
6	Yes	...	1	Right lobe 0.............Left lobe 1
7	Yes	...	1	Right lobe 0.............Left lobe 1
8	...	Yes	2	Right lobe 2.............Left lobe 0
9	Yes	...	1	Lobus Spigelii.
10	...	Yes	17	Right lobe 16.............Left lobe 1
11	...	Yes	Too numerous to count	In both lobes.
12	...	Yes	10	Right lobe 2.............Left lobe 8
13	Yes	...	1	Right lobe 1.............Left lobe 0
14	...	Yes	3	Right lobe 3.............Left lobe 0
15	...	Yes	5	Right lobe 5.............Left lobe 0
16	Yes	...	1	Right lobe 0.............Left lobe 1
17	...	Yes	3	Right lobe 3.............Left lobe 0
18	Yes	...	1	Right lobe 1.............Left lobe 0
19	Yes	...	1	Right lobe 1.............Left lobe 0
20	...	Yes	Very numerous	Right lobe, all but 2
21	Yes	...	1	Right lobe 1.............Left lobe 0
22	...	Yes	30	Right lobe 23.............Left lobe 7
23	Yes	...	4	Right lobe 4.............Left lobe 0
24	Yes	...	1	Right lobe 1.............Left lobe 0

The rupture of a liver abscess is of comparatively frequent occurrence and may take place into the abdominal cavity, the pleura, the pericardium, or into adjoining viscera. Of 24 cases observed at the Army General Hospital, in San Francisco, no less than seven ruptured before death, five into the right pleural cavity, and two into the left pleural cavity and the pericardium. As this question is of considerable surgical importance the following table illustrating the place of rupture in over 100 cases may prove of service:

TABLE III.

ILLUSTRATING THE SITE OF RUPTURE IN AMŒBIC ABSCESS OF THE LIVER.

Observers.	Cases of liver abscess.	Cases of rupture.	Pericardium.	Pleura.	Lung.	Colon.	Stomach.	Bile ducts.	Vena cava.	Kidney.	Lumbar region.
Waring..........	300	68	14	28	15	2	1	1	3	2	2
Dutroulau......	66	25	2	10	7	1	1	..	..	..	4
Rouis...........	162	54	11	17	14	3	6	2			
Haspel.........	25	6	4	2							
Cambay........	10	3	..	2							
Howard........	6	5	5								
Craig...........	24	7	2	5							

When very large or situated near the surface an amœbic abscess is often visible externally, but frequently they are only discovered upon section of the liver; when multiple abscesses are present only one or two may be visible, the others being situated deep within the organ. In such instances a careful examination will always disclose the fact that there is

always one which shows by its size and the thickness of the abscess wall that it is of longer duration than the others.

The contents of an amœbic abscess vary with the character of the infection. In those abscesses in which the amœbæ are present in pure culture, the material contained within them is characteristic, consisting of a semi-fluid, yellowish-red, or chocolate-colored mass, containing shreds of necrotic tissue, blood, a few pus cells, and amœbæ. This material does not resemble pus unless there is a mixed infection with suppurative bacteria, but if such bacteria be present the contents of the liver abscess resemble that of other abscesses due to bacterial agencies.

The character of the abscess wall is typical in those instances in which the amœbæ are present alone. Internally it is covered with shreds of necrotic tissue giving it a peculiar shaggy appearance. When such an abscess is washed out it will sometimes be found that these necrotic shreds reach across it, and I have observed cases in which all trace of liver substance within the abscess had been lost, except the connective tissue framework of the organ which, being more resistent to necrosis than the other elements, still persisted as shreds of tissue crossing the abscess cavity. This typical appearance of the wall is observed most frequently in medium size abscesses, as in the very

old abscesses a mixed infection is generally present and the wall of the cavity is generally smooth or moth-eaten in appearance. In the smaller abscesses the interior is generally almost smooth in appearance, while in the very smallest abscesses no distinct wall can be differentiated.

The appearance presented by sections of the liver abscess under the microscope varies with the age of the process. In the earliest stage, there is simply a collection of leucocytes and connective-tissue cells, with some congestion of the capillaries in the vicinity, and such sections stained by Mallory's method will sometimes show amœbæ, but not as a rule. In those abscesses which have a well-defined wall the centre of the abscess cavity is seen to consist of necrotic epithelium, lymphoid cells, and leucocytes, together with bacteria, and more or less caseous material. Newly formed bile channels are often observed, and examination of the periphery of the small abscesses shows that the process commences in the interlobular areas. The wall of these abscesses is formed by connective tissue considerably infiltrated by leucocytes and young connective-tissue cells, and amœbæ may sometimes be demonstrated. The liver cells in the immediate vicinity are undergoing necrosis and the bile ducts and capillaries are congested, the bile ducts often being obliterated or encroached upon by

the rapidly growing connective tissue. In the large abscesses, which have a rather thick wall, sections show at the inner border of the abscesses more or less necrotic material, while externally the fibrous tissue is very marked, the appearance being that of granulation tissue, the cells of which are mostly uninuclear. More externally is a layer of less dense connective tissue infiltrated with spindle cells and small round connective-tissue cells. This infiltration varies with the age of the abscess. When the fibrous wall is very thick the cellular infiltration is not as great as where the fibrous tissue is of more recent formation.

The amœbæ are found in the abscess wall in the zone of necrosis, generally near the border of the connective-tissue portion of the wall, which is infiltrated by small round cells. From this it will be seen that amœbæ will but seldom be found in the very old abscesses showing little necrosis and a very thick and dense fibrous wall. They are found most commonly in the medium-sized abscesses presenting evidences of marked necrosis of the liver tissue.

In the contents of liver abscesses, which are due wholly to the amœbæ, are found shreds of necrotic tissue, degenerative liver cells, red blood corpuscles, granular material, and amœbæ. It is remarkable how rare are the pus corpuscles which are found in ordinary pus, sometimes the entire field of the micro-

scope showing only from one-half dozen to a dozen. Amœbæ are not always found in the contents of the liver abscesses, especially in the older ones, but generally scrapings from the walls will demonstrate them. In the abscesses in which there is a mixed infection, pus corpuscles are found as in ordinary pus, together with various bacteria, chiefly micrococci.

The contents of the liver abscess may be sterile except for the amœbæ, or there may be a mixed infection with various bacteria. In the smaller abscesses it is generally found that the pus is sterile, while in the large there is generally a mixed infection. There has been considerable discussion as to the relative importance of amœbæ and bacteria in the production of these liver abscesses, some authorities claiming that the condition is produced entirely by the amœbæ, while others insist that it is due to a mixed infection. At the present time, however, it is generally conceded that amœbæ are capable of producing in the liver the characteristic form of abscess which I have described, and that when there is a mixed infection the abscesses lose their characteristic features and resemble those found in other portions of the body. The amœbæ undoubtedly produce the abscesses primarily, but they afterward become infected with other organisms, especially streptococci, staphylococci, and *Bacillus coli communis*. An examination of the contents bac-

teriologically has resulted, in my experience, in about 40 per cent. of the cases showing a mixed infection with some other organism. My observations as regards the bacteriology of the pus in liver abscesses confirms that of many others who have found that in about one-half the cases there is a mixed infection. It is in these cases, also, that we find the yellow or greenish pus, rather than the reddish or chocolate-colored pus which is so typical of amœbic infection. As regards the frequency with which other organisms are found, Futcher investigated 27 cases, in which he found *Staphylococcus aureus* in 6, *Bacillus coli communis* in 5, usually associated with other organisms; *Streptococcus pyogenes* in 3, and *Micrococcus lanceolatus* and *Bacillus pyocyaneus* in one, each; while 12 showed only the amœbæ. Councilman and Lafleur, in 2 cases, found *Bacillus coli communis* in one. Kruse and Pasquale found streptococci in three dysenteric abscesses, staphylococci in two, and bacilli resembling those of typhoid (probably coli communis) in four.

The peculiar form of liver abscess which I have described is associated only with certain forms of dysentery due to infection with pathogenic amœbæ. To one who has had sufficient experience in the study of such forms of dysentery there can be no doubt of the etiologic relationship of the amœbæ to it and

to the liver abscesses occurring in these cases. While liver abscesses occur without the presence of amœbæ, and even in cases of dysentery which are not due to amœbæ, this is no argument against the etiologic importance of the parasite. In my studies of the form of dysentery which is due to infection with the Shiga bacillus, or bacilli belonging to that group, I have observed abscess of the liver, but the abscesses were so different, both in macroscopic and microscopic pathology, from those due to amœbæ that no mistake could be made in distinguishing them.

The question has been raised as to whether an amœbic abscess of the liver ever occurs primarily, no other symptom of amœbic infection having been noticed, and there can be no doubt that a few authentic instances have been reported, such as the case recorded by Buxton in the person of a woman who died at the Philadelphia Hospital. The autopsy showed four large abscesses in the right lobe and one in the left lobe of the liver the pus of each containing amœbæ, but the most careful examination of the intestine showed no evidence whatever of dysenteric infection. Such a case as this, recorded by a competent observer, is conclusive evidence that amœbic abscess of the liver may occur without a pre-existing dysentery. In the vast majority of cases, however, there is a history of dysentery and the abscess occurs

as a secondary condition. It should be remembered in this connection that it is not necessary that well marked symptoms of dysentery be present or that the disease be in an acute stage, for many cases may have entirely recovered from the dysenteric symptoms before the abscess of the liver is discovered or has developed.

The path by which the amœbæ reach the liver from the intestine undoubtedly varies in different patients. They may reach the liver through the blood-vessels, the lymphatics, or through the peritoneum. It is conceded by most authorities that the most probable way is through the portal vein, and in sections of the intestine amœbæ are frequently observed lying in close proximity to the capillaries, and in several instances I have observed them actually within the capillaries. Councilman and others consider that the most common way is through the peritoneal cavity and amœbæ have been demonstrated in the exudate in peritonitis and in the exudate covering the surface of the liver. This path of infection may explain the location of so many liver abscesses at the upper portion of the right lobe and also the occurrence of superficial abscesses.

It is very doubtful if infection often occurs by way of the lymphatics, although amœbæ are frequently noticed in this location in sections of the intestine.

Amœbic abscesses may occur in other regions than the liver, the amœbæ reaching the various organs by way of the blood-vessels. Several authors have reported the finding of amœbæ in the blood, and undoubtedly a few such instances are authentic.

The Production of Dysentery in Susceptible Animals.—Since the first description of amœbæ various observers have succeeded in producing dysentery in susceptible animals by rectal injection, or feeding material containing amœbæ from human sources to such animals. Unfortunately all the work along this line up to the time of Schaudinn's observations is inconclusive as regards the species of amœbæ used in the experiments, for while there is no doubt but that pathogenic amœbæ were employed we are uncertain as to which pathogenic species the experimenter was dealing with, and for this reason these experiments cannot be used as proving the pathogenicity of either *Entamœba histolytica* or *Entamœba tetragena.* In all the early experiments in which positive results were obtained amœbæ were used which answer to the description of the pathogenic species now recognized, and the experiments proved beyond question that it is possible to produce typical amœbic dysentery in susceptible animals with such amœbæ.

Since the observation of Schaudinn several ob-

servers have shown that *Entamœba histolytica* is capable of producing the lesions of dysentery when injected or fed to susceptible animals. A great amount of work has been accomplished by Musgrave, and Musgrave and Clegg, in experimental dysentery, and it is greatly to be regretted that their work cannot be used in proving the pathogenic relation of specific amœbæ to dysentery, for the reason that they failed to recognize species. As both *Entamœba histolytica* and *Entamœba tetragena* occur in the Philippine Islands, these authors may have been dealing with both species, but their results cannot be definitely attributed to either one. They have, however, succeeded in producing typical dysentery lesions in the intestine of monkeys, and abscess of the liver in the same animals.

Schaudinn experimented upon cats and concluded from feeding experiments that only the spores of *Entamœba histolytica* were capable of causing dysentery in these animals. He thus describes his experiments which prove this point. After stating that he obtained his material from a case of dysentery which became infected in China, he says:

"From this case I took a small quantity of feces, divided it into three parts, dried each in the air, and mixed with it sufficient water for about 20 crush

preparations under cover glasses. These preparations were carefully examined, the examination being conducted upon a mechanical stage and requiring many hours. No forms resembling the cysts of *Entamœba coli* were found, but the small spores of *Entamœba histolytica* were noticed in large numbers, but no vegetative organisms could be demonstrated. The cover glasses were then removed, the feces washed with distilled water, and ten such preparations were mixed with enough distilled water to form 1 c.c. of the mixture. The feces of the animal to be experimented upon, a healthy, strong young cat, was carefully examined for amœbæ and none could be demonstrated. To this cat I gave the 1 c.c. preparation mentioned above, mixing it with milk. On the evening of the third day the cat passed bloody mucoid feces and an examination showed the presence of great numbers of typical *Entamœba histolytica.* In the afternoon of the fourth day the cat perished. Dissection showed typical ulcerous dysentery of the large intestine, and immigration of the amœbæ into the epithelium could be easily established.

"I will mention yet another experiment which goes to prove that the permanent spores by themselves are capable of producing a new infection. The feces of the cats developing dysentery contained only vegetable stages of the amœbæ, no spores

being found. When large quantities of the feces were given to a cat it remained well and for four weeks showed no amœbæ in its feces. It was then fed with the remnant of the dried feces used in the first experiment, which contained multitudes of the spores, and after six days the amœbæ began to appear in the feces. Being older and larger than the other cat it proved more resistant to the infection and did not die until two weeks later. The autopsy showed the lesions of typical amœbic dysentery."

These experiments of Schaudinn throw a flood of light upon the interpretation of the negative results of some observers who have worked with *Entamœba histolytica.* It will be remembered that during the active stage of dysentery, when the symptoms are acute, only the vegetative stages of this species occur in the feces, and that these stages do not cause infection, while the spores which are the infective agents in this species only occur when the conditions for vegetative existence are unfavorable, that is, when the healing process has begun. If these points be remembered the negative results obtained by feeding or injection of feces from active cases of dysentery can be explained, as in such cases the feces do not contain the spores. If, however, the feces from cases which are recovering be used for experimental pur-

poses a large proportion of susceptible animals will develop the disease.

The uniformly negative results reported by some authorities are explained by the fact that they were working with the harmless *Entamœba coli* or with feces containing only the vegetative stages of *Entamœba histolytica.*

Personal Observations.—I have already detailed the negative results obtained in kittens by feeding experiments and rectal injection with feces containing the vegetative and encysted stages of *Entamœba coli.* These experiments have been repeated, using the feces of dysenteric patients containing both the vegetative stages and the spores of *Entamœba histolytica,* with the result that 66 per cent. of the kittens experimented upon by feeding developed typical amœbic dysentery, while 50 per cent. of those in which rectal injections were used developed the disease. Control tests were made with the bacteria occurring in the feces and with feces containing *Entamœba coli.*

Rectal Injections.—This is the method which has been employed by most investigators of this subject, but it is not as successful as the feeding experiments, only 50 per cent. of the animals experimented upon developing the disease. Half-grown kittens were used, about 5 c.c. of feces containing amœbæ being

injected into the rectum. The negative results may be explained by the absence of the infective spores, for while motile amœbæ were present in all the material used, some of it was not examined for spores, as at the time I was inclined to believe that the vegetative stages of this species could produce infection.

In experimental dysentery produced in kittens by the rectal injection of infected material the lesions tend to be localized in the rectum, and are not so severe as when the infection is acquired through the mouth. The incubation period varies from 6 days to nearly two weeks, being longer on the average than in the feeding experiments. The lesions produced were typical of those occurring in amœbic dysentery in man and varied in extent and severity with the length of time the infection lasted.

It is not necessary at this time to give in detail all of the autopsy records of the experimental work just mentioned, but I shall quote here in full the autopsy record of a case of dysentery produced in a kitten by rectal injection as it is characteristic of the findings obtained in all the cases, the only difference being in the severity and extent of the lesions. This kitten was given a rectal injection of 5 c.c. of feces from a case of dysentery contracted in the Philippine Islands, the date of injection being October 19. Upon

October 30, the animal showed amœbæ in the feces, soon developed a bloody diarrhœa and was killed upon November 21, the infection having lasted approximately 30 days.

Kitten 1.—Body that of a half-grown kitten, very greatly emaciated. The abdomen is greatly distended with gas. The mucous membrane of the anus appears swollen and a considerable amount of blood-stained mucus is adherent to it. The subcutaneous fat has almost entirely disappeared and the muscles appear dry and atrophied. The pleural cavities are free from fluid and the lungs appear normal. The heart is greatly congested and contains red clots in all the chambers. The liver is hypertrophied, deeply congested, and marked albuminoid degeneration is present, but there is no trace of abscess formation. The kidneys are congested and upon section present the usual lesions of an acute parenchymatous nephritis. The omentum contains a small amount of fat and is not inflamed. The bladder is filled with urine.

The intestines are greatly dilated with gas and fluid. Upon external examination the large intestine appears swollen, is grayish in color, with small, darker colored areas scattered along it. Upon opening the large intestine the mucous membrane of the rectum is found considerably swollen and inflamed,

but no ulcerations are present. Above the rectum for a distance of about 10 cm. the mucous membrane is very much swollen and œdematous, bright red in color, and between the folds a considerable amount of pus can be seen. For a distance of about 4 cm. from the upper end of the large intestine, the mucous membrane is inflamed, being red, swollen, and œdematous. In this area there are numerous ulcerations, covered in with bloody mucus; they are of small size, somewhat irregular in shape, and extend, in most instances, to the submucosa, although there are a few which extend to the muscular coat of the intestine; the edges are undermined and many of the ulcers are covered with necrotic tissue, brownish yellow in color, which has to be removed in order to expose them. A few of the ulcers communicate beneath the mucous membrane. The small intestine shows a rather severe acute enteritis and the stomach an acute gastritis.

In this case the greatest number of ulcerations occurred near the ileocæcal valve, while the rectum escaped; this is most unusual, as in the other successful experiments by rectal injection the ulcerations were generally confined to the rectum, at most invading the intestine for a short distance only above the rectum.

At autopsy actively motile *Entamœba histolytica*

could be easily demonstrated in smears made from the intestine, being most numerous where the lesions were most severe, although every part of the large intestine showed infection with this parasite. Smears from the small intestine were negative for amœbæ.

The clinical symptoms in this case were similar to those occurring in all the kittens developing amœbic dysentery and to those occurring in man. The first symptom observed was diarrhœa, the stools being frequent and free from blood at first, but soon becoming mucoid and bloody and containing numerous amœbæ. Fever was generally present and emaciation was rapid. In this kitten the diarrhœa, after persisting for several days, ceased, and a period of constipation intervened, covering two or three days, after which the dysenteric symptoms returned and from that time until the animal was killed the bowel movements varied in number from 6 to 10 a day and emaciation became extreme. Amœbæ could always be demonstrated in the feces after the initial diarrhœa.

The lesions produced in kittens by the rectal injection of material containing *Entamœba histolytica* are perfectly typical of the lesions of amœbic dysentery in man, making allowance, of course, for the lesser extent of the surface involved.

Feeding Experiments.—The most successful results in producing dysentery in kittens with this

species of amœba are obtained by feeding the animals with infected fecal material. With this method I have been successful in producing the disease in 8 out of 12 kittens or 66+ per cent. and in every case have demonstrated *Entamœba histolytica* in the feces and in sections of the diseased intestines. The method of experimentation was as follows:

The kittens were starved for 24 hours, at the end of which time they were given milk containing about 5 c.c. of feces in which both motile amœbæ and spores were present; the animals did not object to taking the mixture if they were kept without food for this length of time, but unless this was done they almost invariably refused the infected milk. After feeding, the animals were placed in cages and carefully observed.

When successful the symptoms consisted of diarrhœa, rapid emaciation, loss of appetite and strength, severe tenesmus, the animals appearing much distressed while voiding the feces, and finally death from exhaustion. The feces were blood-stained, containing much mucus and multitudes of motile amœbæ.

The period of incubation varied from 7 to 11 days, the average being 8 days, so that it may be stated that the period of incubation is shorter in feeding experiments than when the infected material is introduced per rectum. In two of the animals

short periods of constipation occurred after the initial diarrhœa, lasting a day or two, but they were always succeeded by diarrhœa with the passage of typical dysenteric stools.

In order to illustrate the lesions produced in these animals the following autopsy records of two of them are inserted, as these are typical of the lesions observed in the other kittens.

Kitten 3.—This kitten was fed once with feces containing *Entamœba histolytica* and seven days later developed diarrhœa, the feces containing blood and mucus, as well as numerous motile amœbæ. At the end of two weeks it died, having presented severe symptoms of amœbic dysentery during this time.

Autopsy.—Body that of a half-grown kitten, very greatly emaciated. Subcutaneous fat entirely absent, and muscles dry and much atrophied. The abdominal cavity is free from fluid and the intestines appear normal externally. The pleural cavities are free from fluid and the heart and lungs appear normal. The liver is brownish-red in color externally, with irregular yellow mottlings. There is a small abscess present at the dome of the right lobe, measuring 0.25 cm. in diameter, showing very distinctly through the capsule of the organ. Upon section of the liver the cut surface appears greatly congested, the lobules are distinct, and no abscesses are found other than the one

mentioned. The gall bladder appears normal. The kidneys appear enlarged and congested and upon section show an acute congestion, with some thickening of the cortex. Externally the large intestine appeared slightly, if at all, congested, although the walls were markedly thickened. Upon opening the large intestine it was found filled with fecal material mixed with a large amount of pus, and blood-stained mucus. About 1 cm. from the anus, which was blood-stained and covered with mucus, there was an area measuring 4 cm. in length, presenting the typical lesions of amœbic dysentery, as they are observed in man. The entire mucous membrane was swollen, congested, and œdematous. Numerous nodular areas projected into the lumen of the intestine, which, when incised, were found filled with a glairy material containing hundreds of *Entamœba histolytica*. There were also numerous ulcerations, more or less irregular in shape, with thickened and undermined edges; many were covered in with necrotic tissue, which, upon being removed, showed that the floor of the ulcer was formed by the muscular coat of the intestine. Many of these ulcers communicated with one another beneath the mucous membrane, and most of them had penetrated to the muscular coat. The remainder of the large intestine presented numerous ulcerations, typical of those seen in the intestine of

patients who have died of amœbic dysentery. The lesions were most marked just below the ileocæcal valve, where large areas of the mucous membrane had been destroyed, the muscular coat of the intestine being exposed.

Kitten 5.—This kitten was fed with milk containing *Entamœba histolytica* several times before dysentery developed. The period of incubation was eight days from the date of the last feeding, but from that time, until it was killed, three weeks afterward, the animal presented the symptoms of amœbic dysentery, there being gradual loss of appetite, emaciation, and a diarrhœal discharge, containing blood and mucus, with numerous motile *Entamœba histolytica.*

Autopsy.—Body that of a half-grown kitten, much emaciated. Subcutaneous fat entirely absent, and muscles much atrophied. The pleural cavities were free from fluid and the lungs and heart appeared normal save for congestion. Upon opening the abdominal cavity the small intestine appeared congested externally. The liver is hypertrophied and greatly congested. The kidneys are congested and enlarged and upon section showed the lesions of an acute parenchymatous nephritis. The large intestine was dark gray in color externally, and was considerably thickened, especially toward the rectum. Upon opening the intestine it was found to contain much fecal

material, mixed with blood, mucus, and pus. Commencing at the rectum and extending for about half the length of the large intestine, the mucous membrane was greatly swollen, bright red in color, and contained numerous ulcers. The majority of the ulcers were spherical in shape, the edges were undermined and greatly thickened, and many were covered in with necrotic tissue. Upon removing this necrotic material the base of the ulcer is found to be formed by the muscular coat of the intestine. The ulcers present were typical of the amœbic ulcerations seen in the intestine of man in every respect. The remainder of the large intestine was black in color and gangrenous, the mucous membrane having been almost entirely destroyed, exposing the muscular coat throughout this portion of the intestine. About 4 cm. below the ileocæcal valve there was a small perforation measuring about one-sixth cm. in diameter.

I believe that it must be evident to anyone from the autopsy records given that the lesions produced in kittens by feeding them with material containing *Entamœba histolytica* are typical of the lesions of amœbic dysentery in man. The examination of sections of the diseased intestines showed the same microscopic pathology observed in sections from the dysenteric intestine of man, and the amœbæ were demonstrated in the same situations within the in-

testinal coats. The amœbæ observed in the feces and in the sections of intestine presented the morphological characteristics of *Entamœba histolytica* in every case.

Control Experiments.—All the kittens experimented upon were very carefully examined for several days prior to infecting them for the presence of amœbæ, and all were found negative, so that there can be no question of a previous amœbic infection.

That amœbæ produced the lesions of dysentery observed in the kittens, and not the bacteria occurring with the amœbæ in the feces, was proven by using pure cultures of the various bacteria from the feces for feeding and injections, and mixed cultures of all the bacteria that could be cultivated were also used in the same manner, but in no case did diarrhœa or dysentery result. While there were probably bacteria present which could not be cultivated, I do not believe that this militates against the conclusion that *Entamœba histolytica* was the cause of the lesions produced.

That the bacteria occurring in the feces of amœbic dysentery cases are not the cause of the lesions observed in experimental animals was previously proven conclusively by Harris. He injected 4 dogs per rectum with fecal material containing amœbæ and in all of them the injection was followed by dysentery. In 2 cases abscess of the liver was found after death,

the contents of the abscesses containing amœbæ. In order to control his experiments he believed it was necessary to cultivate the bacteria found in the feces of dysenteric patients and to inject these separately and together. Regarding these experiments he says:

" Cultures were made from the feces of the same individuals whose discharges had been used to successively produce dysentery, and these were then injected into the intestines of four puppies. There was absolutely no effect produced. It, therefore, seems unreasonable to conclude that the germ that produces the disease is a bacterium; or, at any rate, it seems fairly certain that it cannot be an organism that develops, or even lives, in the culture media ordinarily employed. As neither of these suppositions appears at all probable, and as the amœba was the only other living organism found in the feces, that was probably absent from the cultures, it seems logical to suppose that this parasite is the cause of any morbid state that the injection of these discharges may give rise to. This view is supported by the fact that the amœbæ are abundantly present in and around the ulcers that are found in the intestines of the dogs suffering from experimental dysentery, and it does not appear unreasonable to say that the proof is now fairly clear that these organisms are in reality the causative agents in chronic dysentery."

In one of the infected kittens amœbic abscess of the liver occurred and the pus from this abscess was sterile except for the presence of a few amœbæ, while these parasites were also demonstrated in sections of the abscess wall. This observation is almost conclusive of the etiological relationship of *Entamœba histolytica* to abscess of the liver complicating dysentery.

Control experiments were also made with *Entamœba coli* with a negative result in every case.

My experiments regarding the production of dysentery in kittens by *Entamœba histolytica* have been confirmed by Werner, working at the Sailors' Hospital in Hamburg. He experimented with two strains of *Entamœba histolytica,* only one of which he found infective. He was able to produce dysentery in cats with this strain, but found that after six passages the organism lost its virulence. The incubation period varied from 4 to 18 days, the average being 9 days. Of six cats infected with this species four died, the duration of the disease varying from 7 to 24 days, the average being 15 days. The animals were infected per rectum. Werner states that the lesions were typical of amœbic dysentery and were always confined to the colon, especially the lower portion. Guinea-pigs and rats were found to be resistant to infection with this parasite.

From the evidence which has been submitted it

appears to me to be impossible to conclude otherwise than that *Entamœba histolytica* is the cause of a form of amœbic dysentery. The character of the lesions present in this condition, the constant association of this species with the lesions, and the production of similar lesions in susceptible animals, with infected material, I consider conclusive proof that this parasite causes amœbic dysentery in man.

Cultivation.—The subject of the cultivation of this species of amœba will be considered in the section dealing with *Entamœba tetragena,* but I will here state that I have tried all the methods recommended by those who claim to have been successful in cultivating these organisms, but have never been able to secure growth upon artificial media. In a recent article Noc appears to have been successful in cultivating an amœba occurring in dysentery in Cochin China, which bears a very close resemblance, as indicated in his description, to *Entamœba histolytica,* or *tetragena,* but up to the present time his observations have not been confirmed.

ENTAMŒBA TETRAGENA. Viereck, 1907.

In 1907, Viereck described an amœba occurring in patients suffering from dysentery contracted in Africa which he considered a new species, and to which he gave the name *Entamœba tetragena.* During the same year an independent description of the

same organism was published by Hartmann and Prowazek, who named it *Entamœba africana.* As the description of Viereck was published before that of Hartmann and Prowazek the name applied to the organism by Viereck must remain as the proper zoölogical name of this species. The observations of the authors mentioned have been confirmed by Bensen and others and it is now generally accepted that this species is the cause of a form of amœbic dysentery.

At the time that I had the opportunity of studying amœbæ in soldiers returning from the Philippines to San Francisco, and in the Philippines, the species now known as *Entamœba tetragena* had not been described. I had several times observed amœbæ which could not be considered typical of either *Entamœba histolytica* or *Entamœba coli* in that they possessed a distinct ectoplasm, a well defined nucleus containing much chromatin, and reproduced by simple division and the formation of cysts containing *four* daughter amœbæ. I observed these organisms both in soldiers suffering from dysentery contracted in the Philippines and in the natives of those islands, and until the description by Viereck of *Entamœba tetragena* I considered them as atypical forms of the other species. Upon looking over my notes of cases observed during the past seven years I find frequent notations of the occurrence of an amœba correspond-

ing with *Entamœba tetragena* in dysenteric patients returning from the Philippine Islands, and during the last winter while demonstrating these parasites to the class at the Army Medical School, my attention was called to amœbæ in one of the specimens which answered to the description of this species. The amœbæ in this instance were obtained from a discharged soldier who had contracted dysentery in the Philippine Islands and who had suffered from many recurrences. This observation taken in conjunction with my previous records upon a similar amœba observed in soldiers returning from the Philippines and in natives of the Philippine Islands convinces me that *Entamœba tetragena* is a not infrequent cause of dysentery in those Islands. I have also found this species in material sent me by Captain Siler of the Medical Corps, U. S. Army, from cases of dysentery originating in Illinois.

Much of the confusion and difficulty regarding the differentiation of *Entamœba coli* and *Entamœba histolytica* has undoubtedly been due to the presence in many cases of this third species which possesses morphological features common to both of the other species. Thus, while *Entamœba histolytica* possesses an ill defined nucleus and does not form cysts as does *Entamœba coli,* yet in certain cases of dysentery amœbæ may be observed in which there is a well de-

fined nucleus, but cysts are formed only differing from those of *Entamœba coli* in the presence of four instead of eight daughter amœbæ. The identification of this latter form as *Entamœba tetragena* explains the apparent contradiction and will, I am sure, render the classification of the amœbæ found in man much less difficult.

GEOGRAPHICAL DISTRIBUTION.—The geographical distribution of *Entamœba tetragena* has not been thoroughly studied. The observations of Viereck and of Hartmann and Prowazek prove that it occurs in East Africa, Farther India, China and probably in other countries of the Far East. My own observations show that it occurs in the Philippine Islands, and it is very probable that it is a frequent cause of dysentery in many of the islands of the Pacific. This species is also found in South America, and it is not unlikely that it may be a frequent cause of dysentery in certain portions of the United States.

MORPHOLOGY.—*Entamœba tetragena* resembles in certain features of its morphology both *coli* and *histolytica*. It consists of a mass of protoplasm containing a well defined nucleus, is actively motile, and reproduces by simple division and by cysts containing four daughter amœbæ. I had an opportunity of studying this organism in feces from the case already mentioned, and can confirm the description given of it by Viereck and Hartmann and Prowazek.

Size.—This parasite is slightly smaller than *Entamœba histolytica* measuring from 15 to 45 microns in diameter, while the cysts measure from 7 to 12 microns in diameter. The size is of no value in the differentiation of the species because the organisms resemble in this respect both *Entamœba coli* and *Entamœba histolytica.*

Shape.—When at rest the organism is spherical in shape, but great variations in its contour are observed when it is in motion.

The Protoplasm.—As in other amœbæ the appearance of the protoplasm varies with the age of the organism. The very young amœbæ show a distinctly granular protoplasm in which there is a well defined nucleus. In the older amœbæ the protoplasm appears still more granular and in almost every instance a definite nucleus having a strong nuclear membrane is observed. The ectoplasm cannot be distinguished when the parasite is motionless, but it is very distinct when motility is present.

The Cytoplasm.—When fully developed the cytoplasm of *Entamœba tetragena* presents two well defined portions, an outer, the ectoplasm, and an inner, the endoplasm. This distinction is not evident unless the organism is moving, but in such instances it will be observed that the pseudopodia formed of ectoplasm are composed of material distinctly different

from that composing the endoplasm. The ectoplasm is hyaline in appearance and resembles that of *Entamœba histolytica,* being very refractile and glass-like. I have not been able to distinguish any difference in the appearance of the ectoplasm of this species and that of *histolytica.* Under high power the ectoplasm appears to be composed of multitudes of minute granules suspended in a homogeneous plastic substance.

The endoplasm of this species has a grayish appearance and in the fully developed organism appears to be composed of various sized granules and contains within it bacteria, crystals, and in most instances, one or more vacuoles. In this species the number of vacuoles is not as great as in *Entamœba histolytica,* and not infrequently organisms are observed in which no vacuoles are present. The vacuoles are not contractile so far as I have been able to observe.

The Nucleus.—The nucleus of this species is one of its most characteristic features. It is comparatively large and is always well defined. The structure may be described as follows: Externally there is a well marked nuclear membrane, sharply distinguished from the endoplasm, and very refractile; internally there is a large amount of chromatin situated upon the inner side of the nuclear membrane

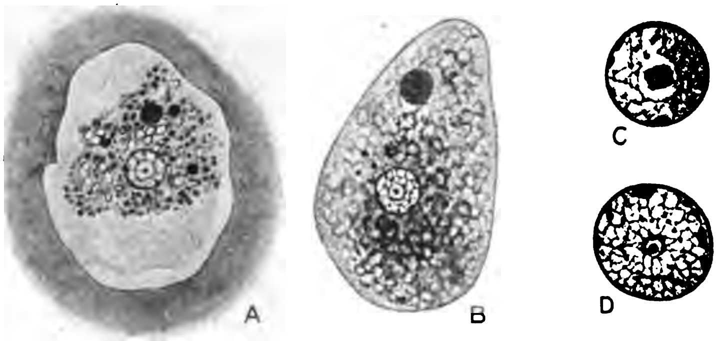


FIG. XXII.—*A*, *Entamœba tetragena.* Living vegetative form showing character of the nucleus and the well-defined ectoplasm. (After Hartmann.) *B*, *Entamœba tetragena*, stained, showing nuclear membrane, karyosome, and centriole. × 1400. (After Hartmann.) *C* and *D*, two nuclei of *Entamœba tetragena* greatly enlarged, showing the centriole and karyosome and the reticulative appearance due to cyclical changes in the latter. (After Hartmann.)

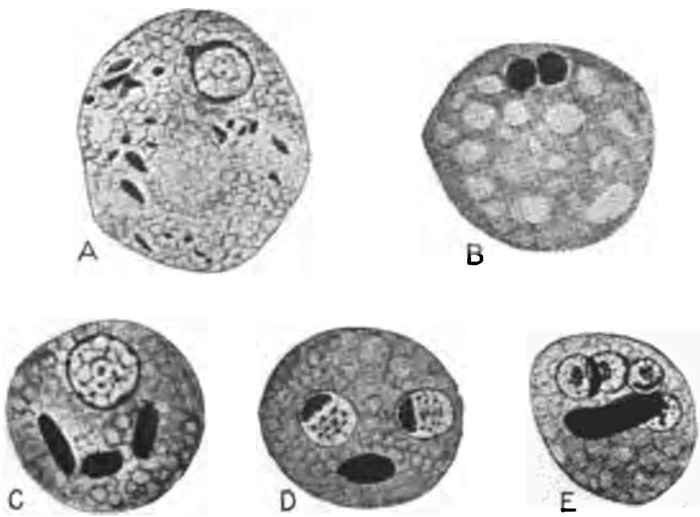


FIG. XXIII.— Various stages in the development of *Entamœba tetragena.* (After Hartmann.) *A*, a parasite showing chromidia and beginning division of the nucleus, the centriole having disappeared; *B*, organism showing division of the nucleus; *C*, a cystic form in the uninuclear stage; *D*, encysted form containing two nuclei; *E*, encysted form containing four nuclei.

in nodular masses and distributed throughout the nuclear substance in the form of a network of minute oval or irregular granules; the karyosome is large and has a centriola or centrosome, surrounded by a clear area, and in some instances two or three concentric clear areas surround the centriola; the latter is spherical in shape and varies in size from a minute dot to a comparatively large spherical mass.

During the reproductive cycle certain changes occur in the karyosome by reason of the rearrangement of the chromatin which lead to marked differences in the appearance of the nucleus at different stages of development.

Vacuoles and Contained Bodies.—Vacuoles are not as constantly present in this species as in *Entamœba histolytica.* Very frequently well-grown organisms are observed in which a vacuole is absent, while they are never as numerous as they may be in the latter species.

This organism is phagocytic for red blood corpuscles and in feces containing blood the amœbæ are usually observed to have engulfed the red cells. So far as I have been able to observe they are as actively phagocytic as *Entamœba histolytica.*

The cytoplasm of this species does not contain the small oval bodies which are so frequently found in *histolytica,* although during certain stages of re-

production the collections of chromatin which form the nuclei of the young amœbæ may appear as round or oval, highly refractile masses, within the endoplasm.

As in other parasitic amœbæ the endoplasm contains bacteria of various kinds, crystals and extraneous matter derived from the feces.

Motility.—What has been said regarding the motility of *Entamœba histolytica* applies equally as well to this species. The same forms of motility are observed and I have not been able to detect any difference in the rate of motility, both of these species being very actively motile as compared with *Entamœba coli.*

The pseudopodia which are projected when the organisms are in motion are composed entirely of ectoplasm and are generally more or less finger-like in shape, having blunt extremities. In this species the pseudopodia are very clearly distinguished from the rest of the organism and are clear and glass-like in appearance. When the organisms are actively motile the endoplasm very quickly flows into the pseudopodia so that in some instances it is rather difficult to distinguish the boundary line between these two portions of the cytoplasm.

Stained Preparations.—This parasite may be stained by any of the methods which have been recom-

mended for staining amœbæ. The Wright stain and the iron hæmatoxylin methods give the best results, and if the latter is used the specimens should be fixed while wet.

Entamœba tetragena does not show the difference in the staining reactions of the ecto- and endoplasm with Wright's stain which is so characteristic of well stained preparations of *Entamœba histolytica.* In this species the cytoplasm stains a well marked blue, when Wright's stain is used, while the nucleus, being rich in chromatin, stains a ruby red or dark violet color. In organisms which are undergoing division the nucleus is often observed to be divided into two spherical reddish bodies connected by delicate pinkish strands of chromatin while further division is sometimes indicated by the presence of three or four masses of red stained chromatin situated within the endoplasm. This species does not show the division of the chromatin into delicate fibriles, distributed throughout the cytoplasm, as does *Entamœba histolytica,* but elongated spindle-shaped masses of this substance are frequently observed in amœbæ undergoing reproduction.

Reproduction.—This species of amœba reproduces by simple division and by cyst formation with the production within the cyst of four young amœbæ.

Simple division is preceded by the mitotic division

of the nucleus, followed by the division of the cytoplasm, resulting eventually in the production of two amœbæ. Mitosis is of a primitive type, but in well stained specimens, especially if the iron hæmatoxylin method be used, mitotic figures are frequently observed. The karyosome first divides, followed by the division of the nucleus.

Reproduction in a cyst is preceded by cyclical changes in the nucleus. These changes have been well described by Hartmann, who states that in no other species of amœba has he observed such clear cyclical changes in the karyosome. Wet-fixed preparations, in sublimate alcohol, and stained with iron hæmatoxylin should be used in studying the nuclear changes.

Prior to encystment the karyosome apparently becomes differentiated into distinct portions, a network composed of refractile fibres which contains within it a clear fluid-like substance. The clear area surrounding the centriole gradually disappears and prior to division the nucleus appears to be composed of a delicate network upon which are arranged minute masses of chromatin which appear highly refractile in the living specimen.

As these changes are occurring in the nucleus the organism extrudes all foreign matter, becoming hyaline in appearance and spherical in shape. The nucleus

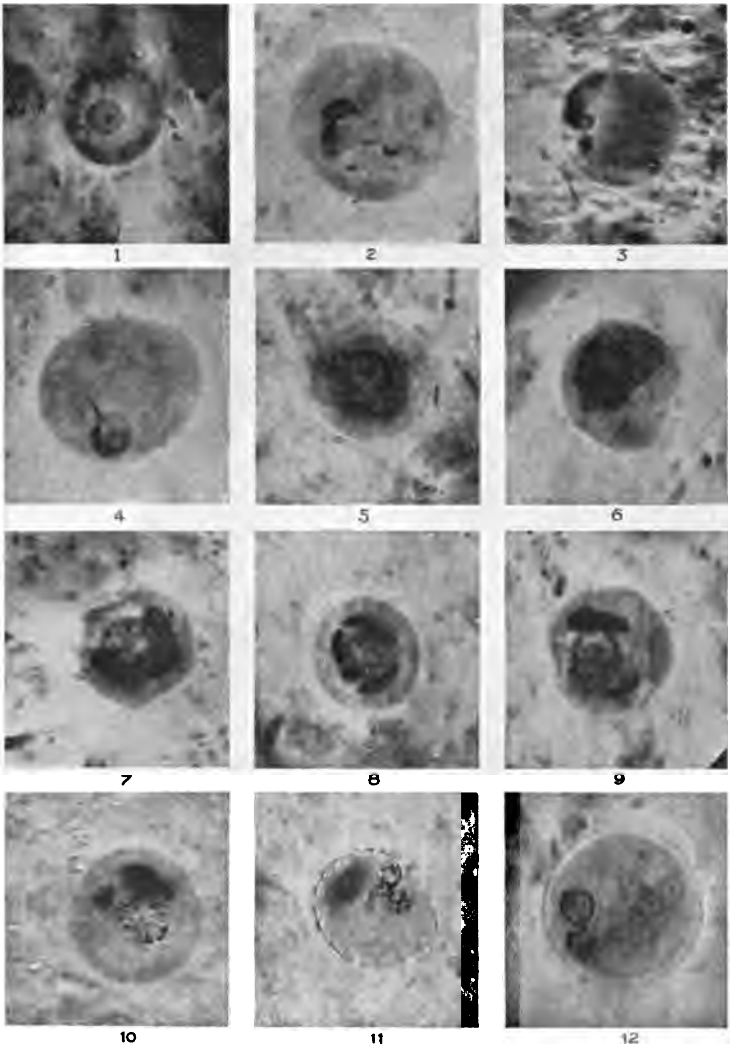


FIG. XXIV.— Various stages in the life-cycle of *Entamœba tetragena*. (After Viereck.) 1, vegetative form; 2 and 3, forms showing division of the nucleus; 4 to 12, stages in nuclear reduction and development. Note the large amount of chromatin during some of the stages. Figs. 11 and 12 are encysted forms.

then divides into two well defined portions, each containing a large mass of chromatin and several small granules of the same substance, the large mass being situated at one side of the nucleus. The chromatin apparently increases in amount during this process of division and some of it is distributed to the cytoplasm. A cyst wall is gradually developed, and the two nuclei each divide, thus forming the nuclei of four young amœbæ.

During this process of reproduction the chromatin at certain stages of development occurs as round or band-like masses, often spindle-shaped, and several such masses may be scattered through the cytoplasm. Later these masses collect into compact groups, some of which take part in the formation of the nuclei of the young amœbæ while some fuse into one or two very compact masses which remain in the cyst as residual bodies.

Conjugation.—I have observed what appears to be conjugation in this species of amœba, the process being similar to that described for *Entamœba histolytica.* The two conjugants appear to fuse together and an interchange of the cytoplasm is probable, as there are well marked streaming movements of the cytoplasm perceptible in the endoplasm of both organisms. In the instances of this process that I have observed these phenomena lasted for

several minutes, after which the conjugants separated, but again became attached and the process was repeated. After about half an hour final separation occurred, the organisms moving off to different portions of the microscopic field.

A similar process has been observed by Werner and he considers that one of the conjugants appears clearer than the other and that it is possible to thus distinguish them.

Cultivation.—Attempts to cultivate *Entamœba tetragena* have always resulted in failure, although the most careful work upon this subject has been done by Viereck, Hartmann and Prowazek, and Werner. Regarding cultivation, Werner says:

"In no case did I succeed in causing a multiplication of the vegetative forms of *histolytica* or *tetragena* that were present in the infective matter. But on the other hand, I often found growth and encystment of *Amœba limax*, on the culture material, and from Musgrave and Clegg's illustrations I am convinced that these observers, as well as Walker, who obtained his cultures from them, grew nothing but *Amœba limax* on their culture material, and that it is this that they have described. It is certain that by cats, and probably man, the encysted forms of *Amœba limax* are often swallowed with the food, and traverse

the intestines, to be excreted in a condition still capable of development."

The observations of Werner regarding *Amœba limax* are probably true as all the cultivated amœbæ which I have observed have shown a contractile vacuole which is not present in any parasitic amœba in man. The same negative results were obtained by Hartmann and Prowazek, as well as Werner, in the cultivation of *Entamœba histolytica,* so that I believe that it is still doubtful if either of these amœbæ has ever been cultivated.

Relation to Disease.—The experiments of Viereck, Hartmann and Prowazek, and Werner, prove conclusively that *Entamœba tetragena* produces a form of amœbic dysentery, but Hartmann believes that it is not as pathogenic for cats as *Entamœba histolytica.* The incubation period in his experiments varied between 8 and 10 days, and the infection lasted from three weeks to a month. Upon autopsy the cats presented typical lesions of amœbic dysentery and the amœba was found in sections of the intestine.

Werner, at the Sailors' Hospital in Hamburg, worked with five strains of *Entamœba tetragena,* only three of which he found to be pathogenic. One of these was still infective after five, one after three,

and one after one passage through cats, but they all lost their virulence after repeated passage. The incubation period in these animals varied from 5 to 12 days, the average being 7½ days. The duration of the disease varied from 8 to 25 days, the average being 17 days. He did not find any marked differences between the lesions produced by *tetragena* and *histolytica* and he does not believe that the evidence supports the idea that *tetragena* is less pathogenic than *histolytica*.

In one case Werner observed abscess of the liver in a cat following infection with *Entamœba tetragena*. Regarding this case he says:

"A cat that had been infected per rectum with a strain of *tetragena* from the Far East sickened after five days' incubation, and had dysentery symptoms, amœbæ being found in the stools, which contained blood and mucus. After being ill for 12 days it died, and in the lower portion of the colon were found ulcers. In the right lobe of the liver near the anterior surface was found an abscess as large as a hazel-nut. This contained sticky pus, in which amœbæ of the *tetragena* type were found."

I have had no personal experience with the experimental production of dysentery in cats with *Enta-*

mœba tetragena, but I consider the evidence sufficient to prove that this species is capable of causing dysentery in susceptible animals.

THE DIFFERENTIAL DIAGNOSIS OF ENTAMŒBA COLI, ENTAMŒBA HISTOLYTICA, AND ENTAMŒBA TETRAGENA.—As these species are most commonly found in the human intestine it is important that one be able to distinguish between them, especially between the pathogenic *Entamœba histolytica* and *tetragena* and the harmless *Entamœba coli.*

The differentiation of these parasites rests upon the study of their morphology and of their methods of reproduction. From a practical standpoint the diagnosis must be made from the differences in the appearance of the three species as they are observed in the feces, and such a diagnosis can be made, provided one cares to spend the time necessary for this purpose. I do not mean to infer that one who has never studied amœbæ can differentiate between species, but I firmly believe that anyone who has thoroughly studied these organisms will be able to distinguish species if sufficient material is available. One has to have studied free-living forms of amœbæ, as well as the forms occurring in the feces of man and other animals, to be able to easily differentiate species and much of the confusion and inaccuracy which has arisen in the classification and description of the para-

sitic amœbæ of man has been entirely due to the ignorance of investigators regarding the biology of this class of protozoa. It cannot be denied that many observers have mistaken ordinary water amœbæ for parasitic species and thus the literature is filled with contradictory statements and absurd deductions.

The differentiation of the parasitic amœbæ of man, while it requires experience, is not very difficult in most instances, and certainly justifies Schaudinn's classification. In a previous communication I said:

"I am convinced that many cases have been diagnosed amœbic dysentery, which in reality presented the harmless *Entamœba coli* in the feces, this organism being mistaken for *Entamœba histolytica*. This mistake might easily be made in patients suffering from acute enteritis, in which it is more than probable that the majority would present *Entamœba coli* in the feces, and this fact undoubtedly explains the numerous instances of so-called amœbic dysentery with rapid and complete recovery.

"From my experience there is no disease so resistent to treatment and in which a prognosis is so discouraging as amœbic dysentery. Everyone is familiar with the fact that amœbic dysentery recurs even after long periods of time, and it is very important, both to the patient and the physician, to

know absolutely that the disease being treated as amœbic dysentery is in reality due to *Entamœba histolytica,* and that *Entamœba coli* has not been mistaken for this organism."

Since writing the above I have seen no reason for changing my opinion regarding the importance of differentiating the harmless from the pathogenic amœbæ, and I am convinced that scores of patients have been treated weeks and even months for amœbic dysentery when the only organism present was *Entamœba coli.*

It should be remembered that a differential diagnosis of these species does not rest upon the presence of a *single* morphological feature but should only be made after a careful consideration of *all* morphological data as well as the life cycle of the organisms investigated. To illustrate: not every large, motile amœba, without a distinct nucleus, is *Entamœba histolytica,* but if to these characteristics be added very marked motility and a clearly differentiated and highly refractile ectoplasm, we may rest assured that we are dealing with *Entamœba histolytica* and not *Entamœba coli.* Bearing in mind, then, that our differential diagnosis must depend upon the presence of *several* morphological features rather than one, the following may be said to be the chief points in which

these species differ from one another, as observed in fresh preparations.

Cytoplasm.—The very marked distinction between the ectoplasm and the endoplasm in *Entamœba histolytica* and *Entamœba tetragena* is one of the most important features differentiating them from *Entamœba coli.* This distinction can always be made in the motile organisms, and very frequently, in the case of *Entamœba histolytica,* when it is motionless. The ecto- and endoplasm in *Entamœba coli* can hardly be distinguished even when the organism is moving and never present the glass-like appearance observed in the other species.

Nucleus.—In *Entamœba coli* the nucleus is almost always visible, situated near the centre of the organism, and containing much chromatin, while it is bounded by a very thick, well defined nuclear membrane. In *Entamœba histolytica* the nucleus is generally invisible, and when visible, is situated near the periphery of the organism; contains but little chromatin; and has no definite nuclear membrane. In *Entamœba tetragena* the nucleus is generally visible; is large; contains much chromatin; and has a well defined nuclear membrane. It is distinguished from the nucleus of *Entamœba coli* by the broad hyaline area surrounding the centriole and by the cyclical changes occurring in the karyosome during reproduction.

Vacuoles and Contained Bodies.—In *Entamœba histolytica* a vacuole is always present, except in the smallest individuals, and generally there is more than one. This species also contains small oval bodies representing the nuclei of the young spores, and generally one or more red blood cells in cases where the feces contain blood. In *Entamœba coli* a vacuole is generally absent and more than one of rare occurrence, while the oval bodies and red blood cells are not observed. In *Entamœba tetragena* the vacuole is frequently absent, although these bodies are more often present in this species than in *Entamœba coli.* This species may also contain red blood corpuscles when they are present in the feces.

Motility.—In both *Entamœba histolytica* and *Entamœba tetragena* motility is very marked, the organisms progressing quite rapidly in a more or less definite direction. Under the same circumstances *Entamœba coli* is very sluggishly motile, while it is almost never seen to progress in a definite direction. This feature alone will serve to distinguish *coli* from the pathogenic amœbæ if the organisms are observed in freshly voided feces, for it will invariably be found that amœbæ exhibiting marked motility will present morphological features proving that they belong to the pathogenic species.

To recapitulate: if in a freshly voided specimen

of feces we observe amœbæ showing sluggish motility, no distinction between the ecto- and endoplasm, or a very slight distinction, and the presence of a nucleus having a well defined nuclear membrane and containing much chromatin, we may diagnose the organism as *Entamœba coli;* under the same conditions, if we observe an amœba which is actively motile, presents a clear, glass-like ectoplasm sharply distinguished from the endoplasm, and a nucleus having a well defined nuclear membrane and a clear area surrounding the centriole, the diagnosis will be *Entamœba tetragena;* finally, if an actively motile amœba is observed, showing a clear distinction between the ecto- and endoplasm (the former being clear and glass-like in appearance) while a nucleus is absent, or if present, shows no nuclear membrane and but little chromatin, the diagnosis will be *Entamœba histolytica.*

Stained Preparations.—In well stained preparations with Wright's stain the three species may be distinguished by the staining of the ecto- and endoplasm. In *histolytica* the ectoplasm stains more intensely than does the endoplasm, while the opposite is true in *Entamœba coli.* In *Entamœba tetragena* there is but little distinction between the staining reaction of the ecto- and endoplasm. In stained specimens showing the reproductive forms the various

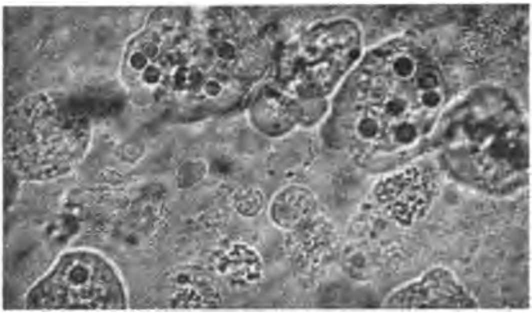


FIG. XXV.—Several vegetative forms of *Entamœba tetragena*, some of which contain numerous red blood-corpuscles. (After Viereck.)

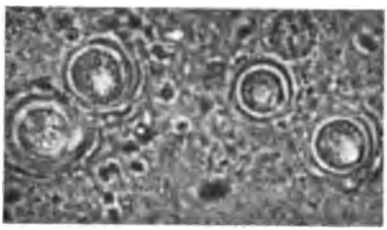


FIG. XXVI.—Encysted forms of *Amœba limax*, which are often mistaken for those of *Entamœba histolytica* and *Entamœba coli*.

species may be distinguished by the amount and arrangement of the nuclear chromatin, as has already been described.

METHOD OF REPRODUCTION.—To one who cares to devote the time and study necessary, the investigation of the methods of reproduction of the species under discussion will definitely distinguish them. While they all reproduce by simple division when conditions are favorable to vegetative existence, they differ widely in their methods of reproduction under other conditions. *Entamœba histolytica* reproduces by spore formation, the young spores being budded off from the periphery of the mother organism, a method totally different from that of *Entamœba coli* which encysts, eight daughter amœbæ being formed within the cyst. Under the same conditions *Entamœba tetragena* undergoes encystment, but only four daughter amœbæ are formed within the cyst. At a certain stage in the development of *Entamœba coli* the cyst contains only four nuclei and care must be taken not to mistake this stage for the cysts of *Entamœba tetragena*. These differences in the reproductive cycle are of the greatest importance in the differentiation of species and whenever there is any doubt concerning the nature of an amœba present in a given case the methods of reproduction should always be studied.

In order to facilitate the diagnosis of the three species under discussion I have prepared Table IV, giving the chief differential points between them.

ENTAMŒBA MINUTA. Elmassian, 1909.

Elmassian has described an amœba in cases of dysentery occurring in Paraguay which he considers a new species and to which he has given the name *Entamœba minuta* because of its small size, specimens seldom exceeding 14 microns in diameter.

Geographical Distribution.—So far as is known this species occurs only in South America, and at the present writing has been described as occurring in Paraguay, but will probably be found distributed throughout South America, and the adjacent islands.

Morphology.—*Entamœba minuta* closely resembles morphologically both *Entamœba histolytica* and *Entamœba tetragena,* but is distinguished from them by its small size. From Elmassian's description it would appear that this feature is of the greatest importance in the differentiation of the species and if his observations are confirmed the question of the size of an amœba, hitherto considered as of slight specific importance, will prove to be, in this instance, at least, a valuable distinguishing feature. The description which follows is compiled from Elmassian's

paper, as I have had no personal experience with this species of amœba.

Size.—Elmassian states that the small size of this organism is distinctive. The average measurement is 12 to 14 microns in diameter, but sometimes the organism may measure as much as 16 or 18 microns. In one instance Elmassian observed an amœba measuring 20 microns in diameter, but he considered that it probably belonged to another species.

Shape.—When motionless this parasite is spherical, or slightly oval in shape, but when moving many variations occur in the shape of the organism due to the extrusion of pseudopodia.

The Protoplasm.—The protoplasm consists of an alveolar structure containing a nucleus which is situated near the centre of the organism. A vacuole is often present and, when blood is present in the feces, the amœbæ frequently contain red blood corpuscles.

The Cytoplasm.—The cytoplasm of *Entamœba minuta* is divided into a well marked ecto- and endoplasm, but these two portions cannot be distinguished unless the organism is moving. The ectoplasm is clear and very refractive in appearance, while the endoplasm is granular in structure and grayish in color. Under high power the endoplasm appears to be composed of multitudes of granules arranged in an alveolar ground substance.

TABLE

DIFFERENTIAL FEATURES OF ENTAMŒBA COLI,

Name.	Size.	Pseudopodia.	Motility.	Protoplasm.
Entamœba coli .... Schaudinn, 1903.	10 to 30 microns. Generally smaller than *Entamœba histolytica* or *Entamœba tetragena*.	Small, blunt, and not clearly differentiated from rest of parasite.	Sluggish	Ectoplasm not distinct except when moving and then only because it is free from granules. Is grayish in color and not very refractive. Endoplasm is gray, finely granular, few non-contractile vacuoles. Is not generally phagocytic for red blood corpuscles.
Entamœba histolytica ..... Schaudinn, 1903.	10 to 70 microns. Generally from 15 to 40 microns.	Blunt or slender and finger-shaped. Very refractive and clearly differentiated from rest of the parasite.	Active ..	Ectoplasm is very distinct and refractive, in some instances even when motionless. Glassy appearing. Endoplasm is granular, contains numerous non-contractile vacuoles and red blood corpuscles when latter are present in the feces.
Entamœba tetragena...... Viereck, 1907.	10 to 50 microns. About the size of *Entamœba histolytica*.	Lobose or finger-shaped. Very refractive and well differentiated from rest of parasite.	Active ..	Ectoplasm and endoplasm well differentiated. Ectoplasm hyaline in appearance. Endoplasm granular, containing numerous non-contractile vacuoles and red blood corpuscles when latter are present in the feces.

IV.

ENTAMŒBA HISTOLYTICA, AND ENTAMŒBA TETRAGENA.

Nucleus.	Cyst Formation.	Cultivation.	Methods of Reproduction.	Pathogenesis.	Staining.
Distinct, having a well defined nuclear membrane and much chromatin. Large karyosome.	Present. Eight young amœbæ developed within cyst.	Doubtful	By simple division, autogamous sexual reproduction in cyst, and by schisogony with the production of eight daughter amœbæ. Eight amœbæ are produced within the cyst.	Is not pathogenic, occurring in a large percentage of healthy individuals, and in patients suffering from diseases other than dysentery.	With Wright's stain, ectoplasm light blue, endoplasm dark blue, and chromatin of nucleus, red.
Indistinct. No well defined nuclear membrane and but little chromatin. Minute karyosome.	Minute spores developed by budding from the parent organism. Measure from 3 to 5 microns. Are covered with a cyst-like membrane.	Doubtful	By simple division, gemmation, and by the budding of chromidial masses surrounded by protoplasm from the periphery of the mother parasite, forming the so-called cystic spores.	Is the cause of a form of amœbic dysentery.	With Wright's stain, ectoplasm dark blue, endoplasm light blue, and chromatin of the nucleus pale red or pink.
Distinct, having definite nuclear membrane formed by chromatin. Large karyosome. Clear area surrounding the centriole.	Present. Four amœbæ develop within cyst.	Negative	By simple division, and by autogamous sexual reproduction within cyst, four amœbæ being produced.	Is the cause of a form of amœbic dysentery.	Does not stain well with Wright's stain. Chromatin takes a bright red stain.

The Nucleus.—This structure is always well defined and is remarkable for its richness in chromatin and for its regularly spherical form. A minute karyosome can be distinguished containing a collection of fine granules which are situated at the centre, forming a centriole. In the vegetative stage the diameter of the nucleus does not exceed 2½ to 3 microns, but in the cystic stage the diameter of the nucleus is from 4 to 6 microns. The chromatin is collected at the periphery of the nucleus, thus giving rise to a very solid, refractive nuclear membrane. During reproduction the chromatin is scattered throughout the nuclear substance and becomes arranged in threads and granules. In some instances a wreath of chromatin granules may be observed surrounding the minute centriole.

Vacuoles and Contained Bodies.—A contractile vacuole has not been observed in this species, and very frequently vacuoles are absent, even in well developed organisms. A single vacuole is most frequently observed, but there may be two or three, especially in organisms undergoing division. This species is phagocytic for red corpuscles and also for certain parasites occurring in the feces. Elmassian states that he has observed *Chlamydophrys stercorea* within amœbæ of this species. Like other amœbæ, this organism often contains bacteria and crystals of various kinds.

Motility.—Entamœba minuta is actively motile, the pseudopodia being composed entirely of ectoplasm. It is only in the moving organism that the ectoplasm can be distinguished and it resembles that of *histolytica* and *tetragena,* being clear and glass-like in appearance. The pseudopodia are generally short and blunt, but may sometimes be finger-shaped, especially in the more actively motile amœbæ.

Reproduction.—The reproductive processes in this species are very similar to those occurring in *Entamœba tetragena,* consisting of simple division, schizogony, and reproduction within a cyst.

Simple division is preceded by the mitotic division of the nucleus after which the cytoplasm divides, two amœbæ being produced. The process is similar to that in other amœbæ, and has been described in preceding sections.

Schizogony results in the division of the organism into four young amœbæ. It is initiated by the division of the nucleus by mitosis into four portions and is completed by the division of the cytoplasm. During the division of the nucleus a well marked equatorial plate is frequently observed.

Reproduction within a cyst is initiated by the extrusion from the organism of all foreign particles, the cytoplasm becoming clear in appearance, while the nucleus increases in size to twice its former

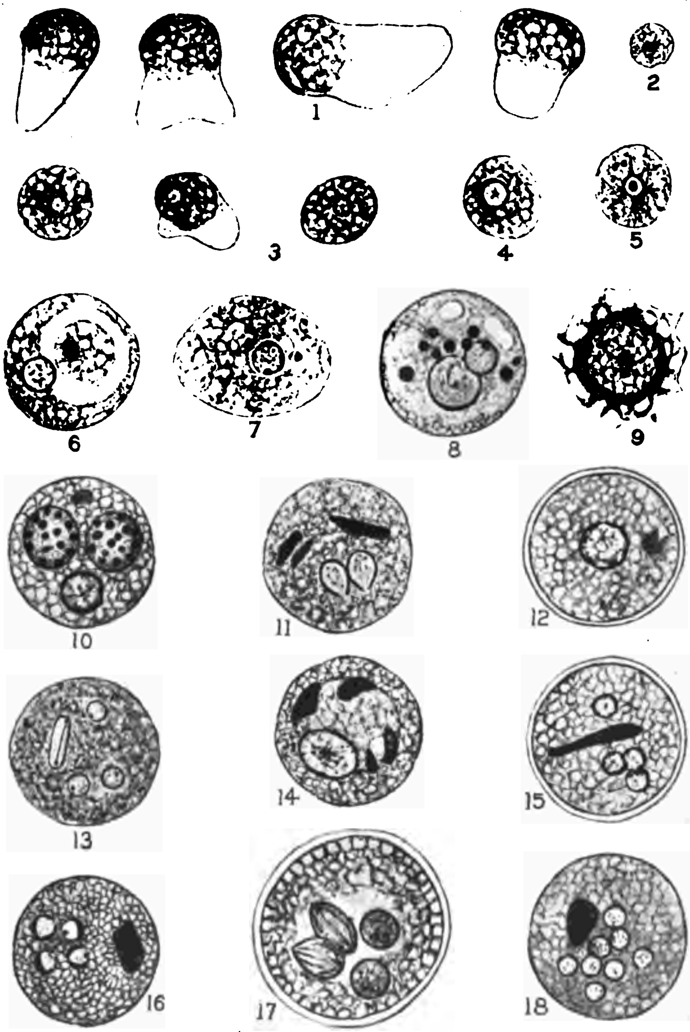
diameter. The chromatin becomes arranged in semilunar bands crossing the nucleus and eventually an equatorial plate is formed, after which the nucleus divides into two equal portions. The nuclei now undergo similar changes to those described in autogamie in *Entamœba coli* and finally four nuclei are produced representing the nuclei of the four daughter amœbæ.

The cysts are generally spherical in shape and measure 12 microns in diameter, although some are observed as small as 10 microns and as large as 14 microns. During the process of encystment a gelatinous membrane is formed having a double outline. The protoplasm of the cyst at certain stages of development appears reticular, the alveoli being minute in size. Sometimes cysts are observed containing three nuclei, but Elmassian considers that these are degenerating organisms.

Relation to Disease.—Elmassian does not give any data regarding the experimental production of dysentery in animals with material containing this species of amœbæ, basing his belief in the pathogenic nature of the organism upon its constant occurrence in the feces of patients suffering from diarrhœa and dysentery. From his description it is apparent that this parasite bears a very close resemblance in morphology and methods of reproduction to *Entamœba*

FIG. XXVII.—Various stages in the life history of *Entamœba minuta*. (After Elmassian.) 1, vegetative forms which are motile, showing the distinction between the ecto- and endoplasm and the lack of a visible nucleus. Note the markedly lobose pseudopodium; 2 and 5, *Chlamydophrys stercoria*, a parasite which is often found within *Entamœba minuta;* 3, motile and immotile forms of *Entamœba minuta*. Note lack of distinction between the ecto- and endoplasm in the motionless forms; 4, young amœbæ produced by schizogony; 6, vegetative form containing a parasite; 7, vegetative form of *Entamœba coli;* 8, encysted amœbæ containing spore-like bodies; 9, encysted *Entamœba minuta*, showing the appearance of the nucleus prior to division; 10, encysted amœbæ containing two other parasites which contain spores; 11 and 12, encysted amœbæ, showing division of the nucleus; 13, encysted *Entamœba coli*, showing division of the nucleus into four parts, one of which shows mitosis; 14, 15, and 16, encysted *Entamœba minuta*, showing division of the nucleus into four daughter-nuclei; 17 and 18, encysted *Entamœba coli*, showing in 17 the four nuclear stage, two of the nuclei undergoing mitosis, and in 18 the eight nuclear stage of this parasite.

Fig. XXVII



tetragena and the fact that the latter species also occurs in South America throws some doubt upon the validity of the species described by this author. The one point of distinction appears to be the uniformly minute size of *Entamœba minuta* and if further observation confirms the statements of Elmassian in this respect it may possibly be accepted as a new species, but it should be remembered that mere difference in size must always be a doubtful feature upon which to base a specific distinction.

ENTAMŒBA NIPPONICA. Koidzumi, 1909.

This species of amœba was described in 1909 by Koidzumi. He observed it first in an advanced case of dysentery in a Japanese in conjunction with *Entamœba histolytica,* but later he found it present in mild cases of the amœbic type of dysentery, and in some cases of bacillary dysentery. He also states that it is not infrequently observed in patients suffering from diarrhœa.

Geographical Distribution.—The geographical distribution of *Entamœba nipponica* is confined to Japan so far as is at present known, but if this be a distinct species it is probable that it will be found widely distributed in the Far East.

Morphology.—In the vegetative stage of development this amœba possesses the general features of the *limax* group, but the pseudopodia are never

spinose. A distinct difference exists in the appearance of the ecto- and endoplasm, while it reproduces by simple division, schizogony, and by spore formation within a cyst.

Size.—Koidzumi gives the average size of this amœba as 25 microns in its longest diameter, but it varies from 20 to 30 microns in length and 15 to 20 microns in breadth. It seldom measures over 40 microns in its longest diameter. There is nothing characteristic about the size of this organism, except that it is longer in one diameter than in the other.

Shape.—When motionless *Entamœba nipponica* is always oval in shape, according to Koidzumi, which differentiates it from the other species of amœbæ which have been described, all of which are spherical in shape when motionless. Numerous changes occur in the shape of the organism during motility due to the extrusion of pseudopodia.

The Cytoplasm.—The cytoplasm of this species much resembles that of *Entamœba tetragena,* being divided into a well marked ecto- and endoplasm. The ectoplasm, however, forms but a very small proportion of the cytoplasm, existing as a narrow rim surrounding the endoplasm. When moving the ectoplasm appears larger in amount because it is concentrated in the pseudopodia. It is clear, colorless and very refractive. The endoplasm, comprising

about seven-eighths of the organism, is light gray in color, very refractive, and granular in structure, the granules being much coarser than in *Entamœba histolytica* or in *Entamœba tetragena.* The endoplasm always contains non-contractile vacuoles of various size.

The Nucleus.—The nucleus is always visible as a well defined spherical body, measuring from 5 to 7 microns in diameter, and situated near the centre of the parasite. A very delicate but distinct nuclear membrane surrounds the nucleus. The chromatin is large in amount and occurs in masses upon the inner surface of the nuclear membrane, there being no chromatin whatever distributed through the nuclear plasma. During the vegetative stage the chromatin appears as refractive masses varying in shape which lie upon, or are attached to, the nuclear membrane. Sometimes they are crescent or spindle shaped and lie lengthwise upon this membrane or they may be attached to it by delicate fibrils. The masses of chromatin vary in number from 3 to 8, according to the age of the parasite, being more numerous in the older organisms.

Vacuoles and Contained Bodies.—A contractile vacuole is not present in this species, but the endoplasm always contains one or more non-contractile vacuoles which vary in size. In cases where the feces

contain blood erythrocytes may be observed within the endoplasm, as this parasite is phagocytic for these cells. Bacteria and other crystals occur within the endoplasm as in other amœbæ.

Motility.—This species is actively motile, the pseudopodia being short and blunt. Koidzumi states that the pseudopodia do not change their shape during motion.

Reproduction.—*Entamœba nipponica* reproduces by simple division, schizogony, and by the formation of daughter amœbæ within a cyst. In reproduction by simple division, Koidzumi states that the nucleus divides amitotically into two portions, followed by the division of the cytoplasm. Simple division has been observed even in the very young amœbæ.

Schizogony is initiated by the crescent or spindle shaped masses of chromatin changing to spheres of nearly equal size, which vary in number from six to eight, and remain attached to the nuclear membrane by a delicate filament. The nuclear membrane gradually disappears and the chromatin masses become free in the cytoplasm. Each mass is then surrounded by a portion of the latter and the entire organism breaks up into as many young amœbæ as there are masses of chromatin. The young amœbæ are oval in shape and measure about 5 microns in diameter. The cytoplasm is dense in appearance and stains

deeply. The nucleus consists at first of a single mass of chromatin, but later a nuclear membrane is developed. The chromatin assumes a crescentic shape and separates into two portions which eventually break up into several small masses situated upon the inner side of the nuclear membrane. While these changes are occurring in the nucleus the cytoplasm becomes differentiated into an ecto- and endoplasm, vacuoles appear, and the organism gradually assumes the appearance typical of the fully developed vegetative stage.

Encystment is first indicated by a reduction in the size of the parasite, although the oval shape is retained. A delicate cyst wall is gradually developed having a single outline. Some of the chromatin masses of the nucleus become spherical in shape and pass through the nuclear membrane into the cytoplasm, where they remain unchanged, so far as he was able to observe. The chromatin remaining in the nucleus divides into minute granules which become evenly distributed over the inner surface of the nuclear membrane. Koidzumi was unable to follow the further development, so that we are in ignorance of the further changes occurring in the nucleus and of the number of daughter amœbæ which are produced within the cyst.

Relation to Disease.—The author gives no data

regarding experiments upon animals with this species of amœba. He believes that it is the cause of a mild form of dysentery in Japan and bases his belief upon the occurrence of the organism in the feces of patients suffering from this type of the disease.

As regards the validity of this species it must be admitted that at this time the data are hardly sufficient to definitely prove that the parasite described by Koidzumi is a distinct species. Its resemblance, according to his description, to both *Entamœba histolytica* and *Entamœba tetragena,* leaves one in doubt as to whether he may not have mistaken certain stages of the development of either of these parasites for a new species.

ENTAMŒBA TROPICALIS. Lesage, 1908.

From his observations Lesage has concluded that the harmless amœba found in healthy individuals and in those suffering from diseases other than dysentery in the Tropics, belongs to a distinct species, and to this parasite he has given the name *Entamœba tropicalis.*

The species resembles *Entamœba coli* in general appearance. It has a distinct nucleus, which contains much chromatin, and reproduces by simple division and by the development of daughter amœbæ within a cyst. It differs from *Entamœba coli* in having a

distinct ectoplasm and by the fact that it can be cultivated in symbiosis with bacteria of various kinds. The cysts are smaller than those of *Entamœba coli* and instead of eight daughter amœbæ being formed within the cyst this species produces from three to as many as thirteen daughter amœbæ.

Lesage believes that this is the species which has been cultivated by Musgrave and Clegg, and that the production of dysentery in animals with such cultures, as reported by the latter observers, was due to contamination with the spores of *Entamœba histolytica.*

According to Lesage, *Entamœba tropicalis* is not pathogenic for animals.

I have not been able to confirm the observations of Lesage regarding the existence of a distinct species of harmless amœba in the tropical regions in which I have studied this subject, and I believe it very doubtful if this species is valid.

ENTAMŒBA PHAGOCYTOIDES. Gauducheau, 1908.

This species was described by Gauducheau, who found it in the feces of a case of dysentery occurring in Indo-China. Its characteristic features are its small size, from two to fifteen microns in diameter; the presence of a well marked ectoplasm; and the fact that it can be easily cultivated on ordinary agar-agar in symbiosis with bacteria. The author gives

no data concerning its relation to dysentery, beyond its occurrence in a single case of this disease. At the present writing his observations have not been confirmed.

ENTAMŒBA UNDULANS. Castellani, 1905.

Castellani describes an organism occurring in the feces of patients in Ceylon, suffering from diarrhœa, which he considers a new species of amœba, and to which he has given the name of *Entamœba undulans.* The organism measures from 25 to 30 microns in diameter, is oval or round in shape and, unlike other amœbæ, it possesses an undulating membrane which is in constant motion. A long narrow pseudopodium is rapidly extruded from the body of the parasite at frequent intervals and is quickly withdrawn, and only one pseudopodium is extruded at a time. The protoplasm is finely granular and there is no distinction between the ecto- and endoplasm. The nucleus is generally invisible and the endoplasm contains a single small vacuole, varying in position. He was unable to observe division and no encysted forms were noticed.

The close resemblance of this parasite to certain stages in the life-cycle of *Trichomonas intestinalis* suggests that the author may have been observing forms of the latter organism, although he states that

the parasite is much larger than *Trichomonas*, and as both it and *Entamœba histolytica* were present in the feces of some of the cases examined, comparison was easy, and he is sure that it did not correspond to either of the latter organisms. His observations have not been confirmed.

PARAMŒBA HOMINIS. Craig, 1906.

In August, 1906, I published the description of a new species of amœba to which I gave the name of *Paramœba hominis*. The genus, *Paramœba*, was established in 1896 by Schaudinn, to include a water amœba which he described at that time. Schaudinn's species occurred in sea water and was peculiar in that a flagellate stage of development alternated with an amœbic stage. The organism described by him, after multiplying for several generations by simple division, at the end of its vegetative life becomes encysted, and within the cyst there develop swarm-spores which are liberated, and after living as flagellates and multiplying by longitudinal division, finally lose their flagellum and again become typical amœbæ. Schaudinn described the process of spore formation as consisting in the division of the nucleus of the encysted amœba, this division being preceded by the division of a cytoplasmic body lying in contact with the nucleus, which acts as a centrosome or blepharo-

plast. The number of spores corresponds to the number of the divisions of the centrosome, each swarm-spore consisting of a portion of the original nucleus and of the cytoplasmic body.

After the formation of the swarm-spores is complete they develop a flagellum, escape from the cyst, and after swimming about actively for an indefinite time, undergo longitudinal division and finally, after losing their flagella, develop into typical amœbæ, which multiply by simple division and again repeat the process of encystment and spore formation.

Schaudinn placed this organism in a new genus, *Paramœba,* and gave it the specific name, *eilhardi.* Until my description the genus was not known to contain any organism living within man.

Paramœba hominis passes through both an amœbic and a flagellate stage during its life-cycle, and for this reason and because the organism morphologically resembled *Paramœba eilhardi,* I had no hesitation in placing it in Schaudinn's genus, *Paramœba.* Doflein is inclined to think that further research will show that the parasite I described should be placed in a new genus, but certainly all the evidence indicates that it belongs to the genus *Paramœba.* The life-cycle is similar in every respect to that of *Paramœba eilhardi* and I am therefore convinced that this parasite is properly placed in the genus *Para-*

mœba, and that this genus must now be regarded as containing a species capable of existing as a parasite in man.

Geographical Distribution.—This species of amœba was first observed in the feces of a Filipino suffering from an attack of chronic diarrhœa and the same organism was afterwards found in the feces of five other Filipinos. At the time that I published my original description, I had never observed the parasite in Americans or Europeans, although I searched very carefully for it, and many of the Americans examined had resided in the Philippines for considerable periods of time.

In 1908, while serving at Fort Leavenworth, Kansas, I was able to study the same parasite in three American soldiers who had just returned from the Philippine Islands, and all of whom entered the hospital because of recurring attacks of diarrhœa.

In all probability these men became infected in the Philippines, and it may well be that this parasite occurs there much more frequently than has been supposed, having been confused with *Entamœba histolytica, Entamœba tetragena, Entamœba coli,* or *Trichomonas hominis.*

So far as the evidence goes, the geographical distribution of this species appears to be confined to the Philippine Islands, but I am of the opinion that care-

ful research will result in proving that the parasite also occurs in this country, for chronic forms of diarrhœa frequently occur in many portions of the United States and monads are frequently reported as occurring in the feces of such cases. It is very easy to confuse the flagellate stage of *Paramœba hominis* with monads unless one is well acquainted with the protozoa occurring in the intestine of man, and I believe that it is not at all unlikely that many of the cases of so-called monadic diarrhœa, or dysentery, are in reality infections with *Paramœba hominis.* It must be admitted, however, that infection with this parasite is rare as compared with the other species of amœbæ infesting man, or with infections with *Trichomonas hominis, Lamblia intestinalis,* or even *Balantidium coli,* for in my own experience, covering the microscopical examination of several thousand specimens of feces from as many individuals, both in health and disease, I have found this organism in only nine patients, three of whom were American soldiers, and six native Filipinos.

MORPHOLOGY AND LIFE-CYCLE.—This parasite has a complicated life-cycle, passing through both an amœbic and a flagellate stage of existence. By making repeated examinations of the feces of infected individuals I have been able to trace the entire life-cycle and while I have been unable to reach definite

conclusions regarding certain points connected with the developmental history, such as the conditions hastening or retarding the various stages of growth, or the intervals of time which elapse between the amœbic and the flagellate stage, and the time consumed in the development of swarm-spores, I have been able to study each stage of development and to confirm in this species the description given by Schaudinn of the life-cycle of *Paramœba eilhardi.*

Beginning with the amœbic stage, the parasite reproduces by simple division for a certain period, probably for as long as conditions are favorable to its vegetative existence, and during this time its structure is that of a typical amœba. Encystment is initiated by the organism becoming motionless, assuming a spherical shape, and then rotating rapidly, the cyst wall being formed during the process of rotation. When encystment is complete, the organism again becomes motionless, and a refractive, double-outlined cyst wall is then distinguishable. Within the cyst there soon appear numerous small, round, refractive bodies, which finally escape from the cyst, each body possessing a single, long flagellum, of very delicate structure. These little flagellates are actively motile, increase considerably in size, and undergo longitudinal division for several generations. At the end of this period of reproduction the para-

sites become motionless; the flagellum disappears; the border of the spherical body remaining begins to undulate; and eventually a blunt, well defined pseudopodium appears, and the parasite enters on its amœbic stage of existence.

The *morphology* of *Paramœba hominis* varies greatly in its different stages of development and for this reason it will be necessary to describe the morphology of each stage, *i.e.,* the amœbic stage, the encysted stage, and the flagellate stage.

The Amœbic Stage.—In this stage the parasites measure from 10 to 25 microns in diameter, the average measurement being from 18 to 20 microns. Amœboid motion is first apparent as an undulatory movement of the periphery of the parasite followed by the projection of small, bluntly conical pseudopodia, in those organisms which have originated from the flagellate forms, but in those amœbæ which result from simple division, amœboid motion is first manifested by the projection of pseudopodia. In the younger organisms amœboid motility is very sluggish, progressive motion being absent, as a rule, although the pseudopodia may be projected and withdrawn with considerable rapidity; in the larger and older amœbæ progressive motion is quite marked, the ectoplasmic pseudopodia being projected rapidly and the endoplasm flowing into them immediately.

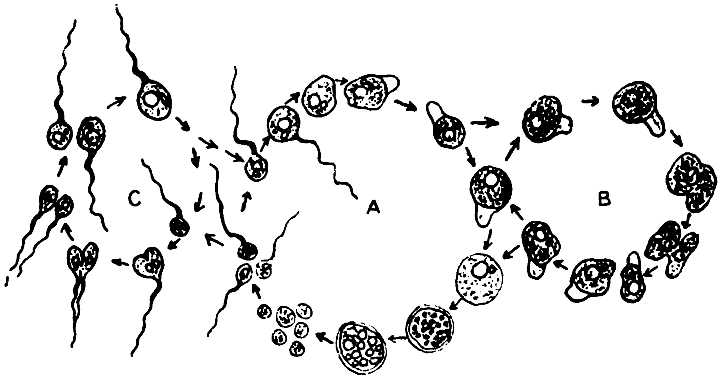


Fig. XXVIII.—Diagram of the life-cycle of *Paramœba hominis*; *A*, the entire life-cycle, showing the amœba and the flagellate stages, as well as the stage of encystment; *B*, reproduction of the amœbic stage by simple division or cycle of reproduction by simple division; *C*, reproduction of the flagellate stage by longitudinal division or cycle of reproduction by longitudinal division.

When motionless no distinction can be made between the ecto- and endoplasm, but when moving, even in the smallest amœbæ, these two divisions of the cytoplasm can be easily distinguished, the endoplasm being more refractive than the ectoplasm and apparently of greater consistence. The endoplasm comprises about three-fourths of the substance of the parasite and is finely granular in structure, while the ectoplasm appears homogeneous in structure and of very slight consistence. The endoplasm may contain bacteria, diatoms, crystals, and occasionally one or more red blood corpuscles.

The greater degree of refraction of the endoplasm of *Paramœba hominis,* as compared with the ectoplasm, serves to distinguish the amœbic stage of this parasite from *Entamœba histolytica* and *Entamœba tetragena,* in which the ectoplasm is more refractive than the endoplasm; and from *Entamœba coli,* in which there is practically no distinction between the ecto- and endoplasm.

The nucleus can be easily distinguished in even the smallest amœbæ; it is a refractive, spherical body, surrounded by a rather thick, very refractive, granular nuclear membrane, which in the larger organisms appears to be composed of brightly refractive rods arranged end-to-end around the periphery of the less refractive nuclear substance, or of large granules

arranged side by side. There is no visible karyosome, but a few very minute granules of chromatin may sometimes be observed in the hyaloplasm. In the fully developed amœbæ an oval body may be observed which lies in contact with, or very near, the nucleus, and which is about one-third the size of the latter. This body undoubtedly corresponds to the cytoplasmic body (*Nebenkörper,* centrosome, or blepharoplast) described by Schaudinn in *Paramœba eilhardi.*

A nutritive vacuole is not present in this species, so far as I have been able to determine, although small oval bodies are sometimes present which suggest vacuoles.

In reproduction by simple division the cytoplasmic body appears to divide first, followed quickly by the division of the nucleus, and finally by the cytoplasm, two daughter amœbæ being thus produced.

In the amœbic stage the parasite stains poorly, although it may be stained with Wright's method, Heidenhain's iron hæmatoxylin, carbol-fuchsin, Borrel blue, or methylene blue. These stains do not differentiate the ecto- and endoplasm, but the nucleus stains fairly well and, when the organism is dividing, shows well marked mitotic figures. With Wright's stain the nucleus appears to be composed almost entirely of chromatin, staining a pink or reddish violet, while the cytoplasmic body may sometimes be dis-

tinguished as a deep violet or almost black mass, lying in contact with the nucleus. In stained specimens the cytoplasmic body is always very small as compared with the nucleus.

The Encysted Stage.—For the observation of the process of encystment it is necessary to examine fresh preparations as the cysts do not stain well.

The organisms which are about to encyst are generally smaller than the average amœbæ, measuring from 15 to 18 microns in diameter, and appear more granular in structure. Amœboid motility is absent and the ecto- and endoplasm are indistinguishable. If one of these organisms be watched it will be observed that it suddenly begins to rotate quite rapidly and that this rotation may last for an hour or more, although it generally ceases within fifteen minutes. The rotation is in one direction and during this process the cyst wall is formed, for when it has ceased it will be observed that the organism is surrounded by a double-outlined, refractive capsule, which sometimes appears slightly mammillated. During rotation the organism contracts somewhat, most of the cysts measuring about 15 microns in diameter.

In the early stage of encystment the nucleus can be distinguished, situated to one side of the centre of the organism, and the cytoplasmic body may also be visible, lying in contact with the nucleus. Both

soon disappear, however, and the cyst becomes filled with refractive granules due to the division of the cytoplasmic body and the nucleus. At a later stage of development the cysts appear to be crowded with small spherical bodies, which are refractive and sometimes appear to move about within the cyst wall, but this motion may be molecular in nature. At the very latest stage of development within the cyst, the outline of the young flagellates may be distinguished, most of them appearing spherical in shape and a dull gray in color, but the flagellum cannot be distinguished while they are still contained within the cyst.

The Flagellate Stage.—I have not been able to observe the escape of the young flagellates from the mother cyst, but groups of these organisms are frequently observed, surrounded by the ruptured cyst wall and arranged in spherical masses corresponding in size with the original cyst. It is very evident that these organisms have developed within the cyst and they very soon assume the typical appearance presented by the flagellate stage of the parasite.

In the youngest stage of development, *i.e.,* just after liberation from the cyst, the swarm-spores do not appear to possess a flagellum; they are very small, measuring from 3 to 6 microns in diameter, are spherical in shape, and have a finely granular

cytoplasm in which the nucleus is not well differentiated. If one watches these young amœbæ it will be observed that they soon become motile, a very delicate flagellum appearing at some portion of the periphery; they disengage themselves from the material in which they often appear to be imbedded, and move about in a rapid, jerky manner, propelled by the flagellum, although at times the latter may appear to draw the organism forward. The very young forms do not stain well with any method which I have tried.

The flagellate forms grow rapidly and when fully developed measure from 10 to 20 microns in diameter. They are spherical in shape except at that portion of the periphery where the flagellum is attached, where the cytoplasm is continued into the flagellum, thus giving the organism a pear-shaped appearance. The flagellum is from three to four times as long as the diameter of the parasite, and tapers very rapidly, the outer three-fourths being so extremely delicate as to require the most careful focussing to demonstrate it. The nucleus in most instances is situated near the origin of the flagellum, which generally appears to be situated posteriorly.

The cytoplasm of the parasite at this stage of development is finely granular in structure and contains a small, well defined nucleus, and a minute

cytoplasmic body, the *Nebenkörper* of Schaudinn. The nucleus is spherical in shape, and has a delicate refractile nuclear membrane. The cytoplasmic body lies in contact with the nucleus, measures about 2 microns in diameter, and is somewhat refractile. In rare instances the cytoplasm may contain one or two red blood corpuscles, so that it is evident that phagocytosis of these cells is not confined to the amœbic stage of development.

Reproduction by longitudinal division may sometimes be observed. When this is occurring the organisms present two nuclei and at certain stages one can observe the partial division of the flagellum into two portions, the splitting of the flagellum being first noticeable at its point of attachment to the body of the parasite. That these forms are not conjugating organisms is proved by the fact that the division of the nucleus may be observed before there is any division of the flagellum, the parasite at this stage showing two nuclei and but one flagellum. It is probable that the division of the nucleus is preceded by the division of the cytoplasmic body, but I have not been able to satisfy myself that this is so. After the division of the nucleus is complete, the cytoplasm, as well as the flagellum, divides longitudinally, thus forming two parasites.

After reproduction in this manner has occurred

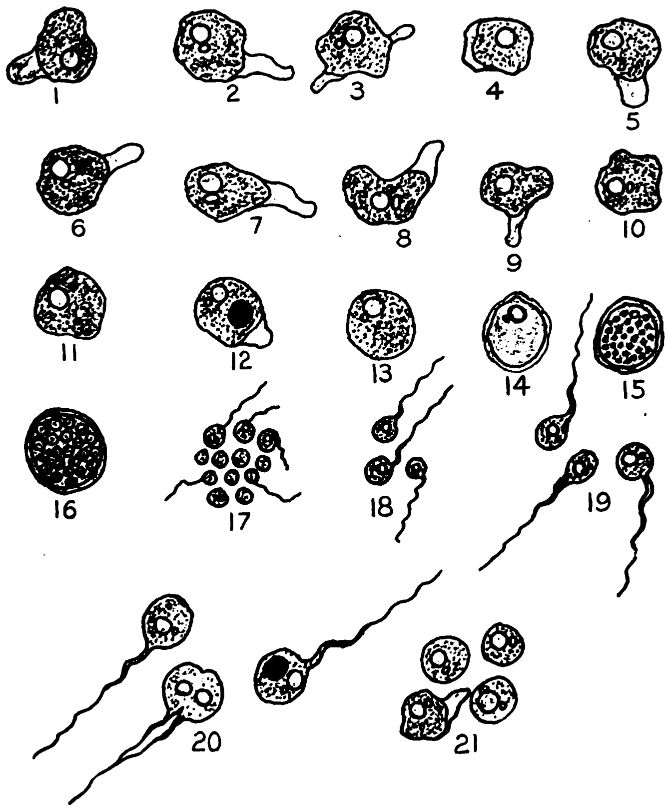


FIG. XXIX.—Various forms of the amœbic, cystic, and flagellate stages of *Paramœba hominis.* 1 to 13, various forms of the amœbic stage of *Paramœba hominis.* Note the well-defined nucleus, the oval or round cytoplasmic body or *Nebenkörper* in contact with the nucleus in some of the forms; the granular protoplasm; the differentiation of the ectoplasm and endoplasm; the blunt pseudopodia. At 12 is shown a form containing a red blood-corpuscle; 14, 15, and 16 are examples of the encysted stage, showing the division of the nucleus and cytoplasmic body into the swarm spores; 17, young parasites just after leaving the cyst. Some show a short flagellum, while others do not show the flagellum; 18 and 19 are developing flagellate forms of *Paramœba hominis,* showing the flagellum, nucleus, and cytoplasmic body; 20, fully developed flagellate forms, one of which is undergoing longitudinal division; 21, flagellate forms just after the disappearance of the flagellum, one of which already shows the typical amœbic stage.

for several generations the parasites become spherical in shape, the flagellum is lost, and the organisms enter upon the amœbic stage of existence.

Even when well developed the flagellate forms stain very poorly, but with Wright's stain I have been able at times to get a clear differentiation between the cytoplasm and the nucleus, the cytoplasm staining a deep blue, while the nucleus stains a reddish violet. With this stain the cytoplasmic body appears almost black in color and is not well differentiated. The flagellum may sometimes be stained a very dim pink, if the stain be allowed to act for an hour or more.

Relation to Disease.—I have not been able to produce an infection in animals with this parasite, but from the clinical symptoms present in the patients infected with *Paramœba hominis* and the fact that recovery quickly follows the disappearance of the parasite in all the cases I have observed, I believe that it is justifiable to conclude that it may cause a form of chronic diarrhœa, characterized by acute exacerbations alternating with periods of constipation. All of the patients were suffering from diarrhœa at the time the parasites were found in their feces, although in one instance the condition was complicated by a severe dysentery due to *Entamœba histolytica*. Omitting this case, we had 8 patients in whom the presence of

Paramœba hominis was accompanied by symptoms of a severe diarrhœa, alternating with periods of constipation. In five of the patients the feces contained a small amount of blood and mucus, while in two *Trichomonas hominis* was present in small numbers. It will thus be seen that in eight of the cases *Paramœba hominis* was the only protozoön present which could be looked on as of possible etiological significance, the two patients showing trichomonads having them in too small numbers to suggest any relation between them and the symptoms present. Treatment by irrigation of the bowel resulted in the disappearance of the parasites, and, with them, of the diarrhœa, and none of the patients have relapsed, so far as I have been able to determine. Thus the clinical evidence points to *Paramœba hominis* as the cause of the diarrhœa, and I believe that we may safely regard this parasite as belonging to the pathogenic protozoa.

Differential Diagnosis.—In the amœbic stage of development *Paramœba hominis* might be confused with *Entamœba coli, Entamœba histolytica,* or *Entamœba tetragena.* If it be remembered that the endoplasm of this species is more refractive than is the ectoplasm, it will be easy to differentiate it from other intestinal amœbæ. However, the occurrence of the peculiar cysts and of the flagellate stage of development, at the same time, should enable one to diag-

nose this species of amœba with but little difficulty. When one observes in feces the peculiar rotating organisms which I have described, one may be sure that *Paramœba hominis* is present.

In the flagellate stage of development the only organism occurring in the feces which might be confused with *Paramœba hominis* is *Trichomonas hominis,* because of certain peculiarities in the development of the latter. *Trichomonas hominis* is frequently observed in the resting stage, when it is spherical in shape, and appears to possess a limited degree of amœboid motion. However, it is much smaller than *Paramœba hominis* and never shows the active progressive motion observed in the latter. The flagellate stage of *Paramœba hominis* is distinguished from active trichomonads by the absence of an undulating membrane, the presence of but one flagellum, and the more spherical form of the organism.

The simultaneous occurrence in the feces of the amœbic stage, the encysted stage, and the flagellate stage of *Paramœba hominis,* is characteristic of this organism, and no difficulty should be experienced by one who is well acquainted with intestinal protozoa, in differentiating this species from other parasites which infest the intestine of man.

VII.

THE AMŒBÆ OF THE MOUTH.

SEVERAL observers have noted the occurrence of amœbæ in the mouth, the parasites being found in the tartar around the roots of the teeth, or in material from the cavities of carious teeth. As long ago as 1862, Steinberg described an amœba in the tartar removed from teeth, to which the name *Amœba buccalis* has been given, and it is more than probable that this species is identical with the one described very fully by Prowazek, who placed it in the genus *Entamœba.*

In 1904 Prowazek investigated the amœbæ occurring in the human mouth and decided that they belong to a distinct species. He gave a detailed description of this parasite and named it *Entamœba buccalis.* He found it present in carious teeth, and considers that all amœbæ which have been described as occurring in the mouth belong to this species.

The GEOGRAPHICAL DISTRIBUTION of this species is world-wide. I have observed it in the mouth of patients in the United States and the Philippines, and it is probable that a careful examination will demonstrate that it occurs in almost every locality.

MORPHOLOGY.—This species is relatively small, measuring from 6 to 32 microns in diameter, the average measurement being 15 microns. It has a distinct ectoplasm, while the endoplasm appears quite granular and is filled with vacuoles. The ectoplasm is seldom visible unless the organism is moving, when it appears clear and hyaline and is little more refractive than the endoplasm. The nucleus is well defined, spherical or oval in shape, and surrounded by a thick greenish nuclear membrane containing much refractive material resembling chromatin. A small centriole, surrounded by a clear area and a small chromatin zone, is situated near the centre of the nucleus.

Motility is sluggish in character and is produced by the extrusion of short, blunt, ectoplasmic pseudopodia into which the endoplasm flows. The motility is not as marked as in *Entamœba histolytica* or *Entamœba tetragena,* but is more marked than in *Entamœba coli.*

Reproduction occurs by simple division, the nucleus dividing by mitosis, and a nuclear spindle is often observed. Schizogony has been described as occurring in this species, the nucleus distributing chromatin to the cytoplasm, which eventually divides into small amœbæ, each containing a small amount of nuclear chromatin.

This organism has not been cultivated artificially so far as I am aware.

There is no experimental evidence connecting *Entamœba buccalis* with disease. It is very doubtful if it has anything to do with caries of the teeth, although it is most frequently encountered in the cavities of carious teeth. However, it may be frequently demonstrated in material scraped from the roots of perfectly normal teeth, so that, as far as the evidence goes, we must regard *Entamœba buccalis* as only a secondary invader of the tissues.

The amœbæ known as *Entamœba gingivalis*, described by Gros, in 1849, and *Entamœba dentalis*, described by Grassi in 1879, are in all probability identical with *Entamœba buccalis* and do not merit a separate description.

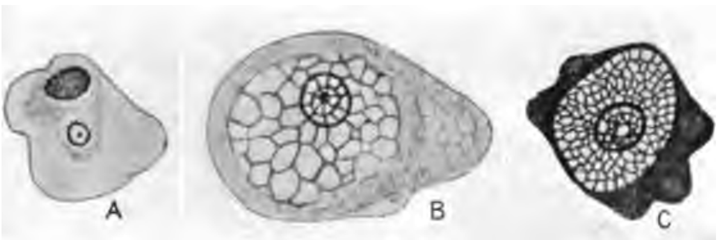


FIG. XXX.—*Entamœba buccalis.* *A*, living form showing the character of the ecto- and endoplasm and the nucleus; *B*, form stained with iron hæmatoxylin, showing the reticulative appearance of the nucleus and endoplasm; *C*, *Entamœba buccalis* stained with the Giemsa stain, showing the reticulative structure of the nucleus and endoplasm. (After Hartmann.)

VIII.

THE AMŒBÆ OF THE GENITO-URINARY TRACT.

In 1883 Baelz described an amœba occurring in bloody urine which measured from 23 to 50 microns in diameter and was actively motile, the pseudopodia being short and blunt. The cytoplasm of this organism was granular in appearance and the parasite was phagocytic for red blood corpuscles. Encysted forms were also observed.

To this organism the name *Entamœba urogenitalis* has been given and Jürgens, Posner, and Kartulis have described similar amœbæ occurring in the urine. It is still undecided whether this organism is entitled to specific rank, most authorities believing that the amœbæ described by these authors were either *Entamœba histolytica* or *Entamœba tetragena.* There is no reason why any of the species occurring in the intestine should not occasionally be found in the urine, reaching it through fistulæ between the bladder and intestine, which may occur in cases of amœbic dysentery, or intestinal amœbæ might reach the kidney or bladder through the surrounding tissues or through the blood stream. I have observed one instance of infection of the bladder with *Entamœba histolytica,* autopsy showing a minute fistula between the ulcerated intestine and the bladder.

IX.

AMŒBÆ OCCURRING IN EXUDATIONS, ABSCESSES, AND IN THE LUNGS.

IJIMA described amœbæ occurring in serous fluid obtained from a woman suffering from peritonitis due to an endothelioma, to which he gave the name of *Entamœba miurai*. He described this organism as measuring from 15 to 18 microns in diameter, and spherical or oval in shape, with a pseudopodium at one end covered with cilia. The cytoplasm is described as finely granular and no distinction was noted between the ectoplasm and endoplasm. From 1 to 3 nuclei were observed, but no data are given regarding the methods of reproduction.

It is generally believed that Ijima mistook cells present in the serous exudation for amœbæ and his observations have not been confirmed. I do not believe that *Entamœba miurai* can be considered a valid species.

In 1892 Flexner described an amœba occurring in the pus of an abscess in the oral cavity, and in 1893 a similar organism was described by Kartulis in abscesses of the lower jaw, and to this parasite the name *Entamœba kartulisi* has been given. The description given by Flexner and Kartulis could be

in part applied to either *Entamœba histolytica* or *Entamœba buccalis,* and it is doubtful if this species can be considered valid. It is well known that under certain conditions *Entamœba histolytica* enters the blood stream and may be carried to distant tissues where it may produce abscess formation and abscesses due to this organism have been found in the brain, lungs and kidneys. From the description given of *Entamœba kartulisi* it is very probable that it is identical with *Entamœba histolytica,* and that the abscesses in which it was found were caused by the latter organism.

Artault, in 1898, described an amœba occurring in the lungs which is now known as *Entamœba pulmonalis.* The description of this species is quite similar to that of *Entamœba histolytica,* and in all probability the latter organism was the one observed by Artault. *Entamœba pulmonalis* cannot be considered a valid species.

REFERENCES

Artault, St. Flore et faune d. cav. pulmon. Amœba pulmonalis, sp. n. Arch. de parasit., 1898, I, p. 275.

Ashburn, P. M., and Craig, C. F. Entamœba coli, etc. The Military Surgeon, 1907, I, p. 21.

Baelz, E. Ueber einige neue Parasiten des Menschen. Berl. klin. Wochenschr., 1883, 10, p. 237.

Bensen, W. Die Darmprotozoon des Menschen. Arch. f. Schiffs- u. Tropen-Hyg., 1908, 12, p. 661.

Berndt. Deutsche Ztschr. f. Chirurgie, 1894, 40, p. 163.

Billet, A. Sur un cas de dysenterie "nostras" a amibes. C. R. Soc. Biol., 1907, lxii, p. 1232.

Blanchard, R. Traité de Zool. médic. T. I. Paris, 1885, p. 33. Idem. Maladies parasit. Paris, 1895, p. 658.

Braun, M. Tierische Parasiten des Menschen. Würzburg, 1908, p. 33.

Braun, M., and Lühe, A. Handbook of Practical Parasitology, New York, 1910.

Brown, W. C. Amœbic or Tropical Dysentery, London, 1910.

Cahen. Deutsche med. Wochenschr., 1891, 17, p. 843.

Calandruccio. Anim. par. dell' nomo in Sicilia. Atti. Accad. Giorn., 1890, 2, p. 95.

Calkins, G. Protozoology. Philadelphia, 1909.

Casagrandi, O., and Barbagallo, P. Recersche Biolog., e cliniche sell' amœba coli (Loesch). Boll. Accad. Groinea. sc. nat. Catania, 1895, xli, pp. 7–17.

Idem. Sui terreni di coltura delle amobe. Ref. med., 1896, 157, p. 7477.

Idem. Entamœbæ hominis a amœba coli (Loesch). Amali d'Igiene sperimentale, 1897, V. I, p. 103.

Idem. Ueber die Kultur von. Amœben. Centralbl. f. Bakt., etc., 1897, 1 Abts. xxi, p. 579.

Castellani, A. Observations on some Protozoa found in Human Fæces. Centralbl. f. Bakt., etc., 1905, 1, xxxviii, p. 66.

Celli, A., and Fiocca, R. Beitrage zur Amœbenforschung. Centralbl. f. Bakt., etc., 1894, 1, xv, p. 470.

Idem. Beitrage zur Amœbenforschung u. d. Klassification der Amœben, etc. Ibid., 1894, 1, xvi, p. 329.

Idem. Richerche interno alla biologia delle amebe. Bull. d. r. Accad. med. di Roma, 1895, xxii, p. 285.

Idem. Ueber die Aetiologie der Dysenterie. Centralbl. f. Bakt., etc., 1895, 1, xvii, p. 309.

Councilman and Lafleur. Amœbic Dysentery. Johns Hopkins Hospital Reports, ii, pp. 395–548, 1891.

Craig, Chas. F. Observations on Amœba coli, etc. Med. News, 1901, lxxviii, 11, p. 414.

Idem. Classification of Amœba coli. Am. Med., Phila., 1904, viii, p. 185.

Idem. The Complications of Amœbic and Specific Dysentery as Observed at Autopsy; an Analysis of One Hundred and Twenty Cases. Am. Jour. Med. Sci., n. ser., 1904, cxxvii, p. 145.

Idem. The Life Cycle of Amœba coli in the Human Body. Am. Med., Phila., 1904, vii, p. 299.

Idem. The Pathology of Chronic Specific Dysentery of Tropical Origin. Jour. Assoc. Mil. Surg., 1904, xiv, p. 353.

Idem. Observations upon Amœbæ Infecting the Human Intestine, with a Description of two Species, Entamœba coli

and Entamœba Dysenteriæ. Am. Med., Phila., 1905, ix, pp. 854; 897; 937.

Idem. The Etiology and Pathology of Amœbic Infection of the Intestine and Liver. Internat. Clin., Phila., 1905, 14 Ser., V. 4, pp. 242–298.

Idem. A New Intestinal Parasite of Man; Paramœba hominis. Am. Jour. Med. Sci., V. Ser., 1906, cxxxii, 8, p. 214.

Idem. Studies upon the Amœbæ in the Intestine of Man. Jour. Infec. Diseases, 1908, 5, 3, pp. 324–377.

Idem. Further Observations on Paramœba hominis, an Intestinal Parasite of Man. Arch. Internal Med., 1910, 6, 1, pp. 1–11.

Idem. Entamœba tetragena as a Cause of Dysentery in the Philippine Islands. Arch. Internal Med., 1911, vii, 3, p. 362.

Cunningham, D. D. On the Development of Certain Microscopic Organisms Occurring in the Intestinal Canal. Quar. Jour. Micro. Sci., 1881, V. Ser. 21, lxxii, p. 234.

Dock, G. Observations on Amœba coli in Dysentery and Abscess of the Liver. Texas Med. Jour., 1890–91, vi, p. 419.

Doflein, F. Lehrbuch der Protozoenkunde. Berlin, 1909, p. 500.

Dopter, C. Sur Quelques Points relatifs a l'action pathogene de l'amibe dysenterique. Ann. Instit. Pasteur, 1905, xix, p. 417.

Idem. Les dysenteries. Paris, 1908.

Dutroleau. Maladies en Pays chauds. Paris, 1868.

Elmassian. Entamœba minuta, etc. Centralbl. f. Bakt., etc., 1909, 1, Abt. Orig. 52, p. 335.

Fantham, H. B. On the Amœbæ Parasitic in the Human Intestine, etc. Ann. Trop. Med. and Parasitology, Ser. T. M. 1911, V. 1, p. 111.

Flexner, S. Dysentery. System of Medicine, Allbutt and Rolleston, Vol. II, Part 2, 1907, London, p. 477.

Frosch, P. Zur Frage der Reinzuechtung der amœben. Centralbl. f. Bakt., etc., 1897, xxi, 24–25, pp. 926–932.

Futcher, T. B. A Study of the Cases of Amœbic Dysentery Occurring at the Johns Hopkins Hospital. Jour. Am. Med. Assoc., 1903, xii, p. 480.

Gasser, J. Note sur les causes de la dysenterie. Arch. Méd. Exp., etc., 1895, ii, p. 198.

Gauducheau, A. An Experimental Production of Amœbic Dysentery, etc. Jour. Trop. Med., 1906, ix, p. 15.

Idem. Note preliminaire sur la culture et la function bacteriolytique d'un protozaire amiboide. Gazette Hebdomadaire d. sci. méd., 1907, 20, 17, p. 193. Also Bull. parasit. Exotique, 1909, ii, p. 247.

Idem. Culture d'une amibe dysenterique (*e. phagocytoides*). C. R. Soc. Biol., 1908, lxiv, p. 493.

Grassi, B. Die Protoi parasite e specialmente di quelli che sono nell' uomo. Gaz. Med. Ital., Lombardia, 1879, p. 445.

Idem. Contributione allo Studio delle amibe. Rendic. d. R. inst. Lombardia, 1881, 2, xiv, p. 353.

Gros, A. Beobacht. uber Amœbenenteritis. Arch. f. klin. Med., 1903, lxxvi, p. 429.

Harris, H. F. Some observations on a Method of Multiplication of the Amœba dysenteriæ (Amœba coli). Med. News, 1894, lxv, 21, p. 567.

Idem. On the Alterations Produced in the Large Intestine of Dogs by the Amœba coli, etc. Hatfield Prize Essay. Phila., 1901.

Idem. Experimentell bei Hunden erzeugte Dysenteria. Arch. Path. Anat. Berl., 1901, clxvi, p. 67.

Idem. Amœbic Dysentery. Am. Jour. Med. Sci., April, 1908.

HARTMANN, M. Eine neue Dysenterie amobe. Beiheft z. Arch. f. Schiffs- u. Tropen Hyg., 1908, v, p. 117.

Idem. Untersuchungen über parasitischen amoben 1. Ent. histolytica Sehandusic. Arch. f. Protistenkunde, 1909, xviii, p. 207.

HARTMANN, M., and PROWAZEK, S. Blepharoplast, Caryosome und Centrosom. Arch. f. Protistenk., 1907, x, p. 312.

HLAVA.—Ueber die Dysenterie. Centralbl. f. Bakt., etc., 1887, 1 Abt. X, p. 537.

IJIMA, J. On a New Rhizopod Parasite of Man. Annot. Zool., Japan, 1898, ii, 3, p. 85. Ref. in Centralbl. f. Bakt., etc., 1899, xxv, p. 885.

JAEGER, H. Untersuchungen u. Amœben-dysenteric in Ostpreussen. Deutsche med. Wochenschr., 1902, 27, p. 208.

JÜRGENS. Zur Kenntniss der Darmamœben und der Amœben-enteritis. Veroff. a. d. Geb. d. Milit-Sanitatswes., Berl., 1902, 20, p. 110.

KARTULIS. Ueber Riesen Amœben bei chromischer Darmentzündung der Aegypten. Virch. Arch. f. path. Anat., 1885, xcix, p. 145.

Idem. Zur Aetiologie der Dysenterie in Aegypten. Virch. Archiv f. path. Anat., 1886, cx, p. 521.

Idem. Zur Aetiologie der Leberabscesse. Centralbl. f. Bakt., etc., 1887, 1 Abt. ii, p. 745.

Idem. Ueber weiters Verbreitungsgebiete der Dysenterie amœben. Ibid., 1890, 1 Abt. vii, p. 54.

Idem. Einiges ueber die Pathogenese des Dysenterie amœben. Ibid., 1891, 1 Abt. ix, p. 365.

Idem. Die amœbendysenterie. Kolle u. Wassermann's Handbuch d. pathogenen Micro-organismen, 1895, 1, 347.

Idem. Dysenterie. Spec. Path. and Flue., Nothnagel. III, Wien, 1896.

Idem. Gehirnabscesse nach dysenterischen Leberabscessen. Centralbl. f. Bakt., etc., 1 Abt. xxxvii, 4, 527.

Koch, R. Arbeit. a. d. Gesondheitsamte. No. 3, 1887.

Koidzumi, M. On a New Parasitic Amœba, Entamœba nipponica. Centralbl. f. Bakt., 1909, 1, Orig. li, p. 650.

Kovacs, F. Beob. u. vers. ut d. sog. Amœben-Dysenteric. Zeitschr. f. Heilkde., 1892, xiii, p. 509.

Kruse, W., and Pasquale, A. Studium des Dysenterie und d. Leberabscesse. Deutsche med. Wochenschr., 1893, 15, 1, 354; 16, p. 368.

Idem. Unters. ueber Dysenterie u. Leberabscesse. Zeitschr. f. Hyg., 1894, xvi, pp. 1–149.

Lambl. Beob. u. Studien aus dem Franz-Joseph-Kinderspital in Prag, 1860, i, p. 362.

Lesage, A. Culture de l'amibe de la dysenterie des pays chauds. Ann. Inst. Pasteur, 1905, xviii, pp. 9–16.

Idem. Culture du parasite de l'amibiase, humaine. C. R. Soc. Biol., 1909, lxii, p. 1157.

Idem. L'amibiase chez le chat. C. R. Soc. Biol., 1909, lxii, p. 1191.

Idem. Note sur l'Entamibe d. la dysenterie amibiene, etc. Bull. d. l'Soc. d. Path. Exotique, 1908, 1, p. 104.

LEWIS and CUNNINGHAM. Ann. Rep. Sanit. Commis. Govt. of India. Calcutta, 1870.

LOESCH, F. Massenk. Entw. v. Amœben in Dickdarm. Virch. Arch. f. path. Anat., 1875, lxv, p. 196.

MARCHOUX. Note sur la dysenterie. C. R. Soc. Biol., Paris, 1899, 1, p. 870.

MARSHALL. The Amœba dysenteriæ. Brit. M. Jour., 1899, 1, 1386.

MASSIUTIN. Ueber die Amœben als Parasiten des Dickdarms. Vratch, 1889, x, 25; Centralbl. f. Bakt., 1889, 1 Abt. vi, p. 451.

MINCHIN. The Protozoa. System of Medicine, Allbutt and Rolleston, London, 1907, Vol. 2, Part II.

MIURA, K. Mitteilg. d. me Fakult. d. kaiserl. Japan Univ. Tokio, 1900, 1, pp. 1–18.

MOUTON, H. Recherches sur la digestion chez les amibes, etc. Ann. Inst. Pasteur, 1902, xvi, p. 457.

MUSGRAVE, W. E., and CLEGG, M. T. Amebas; their Cultivation and Etiological Significance. Bureau of Govt. Lab. Biological Lab., 1904, 18, Manila, P. I.

Idem. The Cultivation and Pathogenesis of Amœbæ. Phil. Jour. Sci., 1906, 1, p. 909.

NAKAGAWA. Endemic Dysentery in Formosa. Mitteil. de med. Gesell., Tokio, 1907.

NOC, F. Sur la Dysenterie Amibiene en Cochinchine. Ann. Inst. Pasteur, 1909, xxiii, p. 177.

NORMAND. Note sur deux cas de colite parasite. Arch. méd. navale, 1879, xxxii, p. 211.

Osler, W. On Amœbic Abscess of the Liver. Med. News, 1902, 15, p. 673.

Patterson, H. S. Endemic Amœbic Dysentery in New York, with a Review of its Distribution in North America. Am. Jour. Med. Sci., 1909, cxxxviii, p. 198.

Pfuhl. Archives de Mèdicine Navale, 1906, p. 401.

Plehn, A. Die Dysenterie in Kamerun. Arch. f. Schiffs- u. Tropen-Hyg., 1898, p. 125.

Prowazek, S. Entamœba buccalis. n. sp. Arb. a. d. kaiserl. Gesundheitsamte, 1904, xxi, p. 42.

Quincke and Roos. Ueber amœben-Enteritis. Berl. klin. Wochenschr., 1893, xxx, p. 1089.

Rogers, L. Tropical or Amœbic Abscess of the Liver, etc. Brit. Med. Jour., 1902, No. 2177, p. 844.

Ruge, A. Amœbenruhr. Handbook d. Tropsmkrank. Mense, 1906, iii, p. 1.

Schardinger. Protozoenkulturen. Centralbl. f. Bakt., etc., 1897, 1 Abt. xxii, p. 3.

Schaudinn, F. Ueber den Zengungskreis von Paramœba eilhardi; neue Genus, neue Species. Sitzungst. d. h. preuss. akad. d. Wissensch., Berlin, 1896, p. 31.

Idem. Untersuch. ueber d. Fortplanzung. d. Rhizopoden. Art. a. d. Kaiserl. Gesundheitsamte, 1903, xix, 3, p. 547.

Schuberg, A. Die parasit. Amœben d. menschl. Darms. Centralbl. f. Bakt., 1893, xiii, p. 598; p. 654; p. 701.

Stiles, C. W. Report on Classification of Amœba coli. Am. Public Health Assoc. Rep., 1905, xxx, p. 292.

STRONG, R., and MUSGRAVE, W. E. Etiology of the Dysenteries of Manila. Ann. Rep. Surg. General, U. S. Army, 1900, p. 251.

STRONG, R. P. Amœbic Dysentery. "Modern Medicine," Osler, Vol. I. Phila., 1907.

TSUJITANI. Ueber die Reinkultur des Amœben. Centralbl. f. Bakt., etc., 1898, 1 Abt. xxiv, p. 666.

VEDDER, E. B. An Examination of the Stools of One Hundred Healthy Individuals, with Special Reference to the Presence of Entamœba coli. Jour. Am. Med. Assoc., 1906, xxvi, p. 870.

Idem. Is the Distinction between E. coli and E. dysenteriæ valid? Jour. Trop. Med., 1907, x, p. 190.

Idem. Efficacy of the Ipecac Treatment of Dysentery. Bull. Manila Med. Soc., 1911, III, 3, p. 48.

VIERECK, H. Studien ueber die in den Tropen erworbene Dysenterie. Archiv f. Schiffs- u. Tropen-Hyg., 1907, Bd. xi, Beihest I, pp. 1–41.

WALKER, E. L. The Parasitic Amœbæ of the Intestinal Tract of Man and Other Animals. Jour. Med. Research, 1908, xvii, p. 379.

WENYON, C. M. Reports Wellcome Research Laboratories, 1908, iii, p. 122.

WERNER, H. Studies Regarding Pathogenic Amœbæ. Indian Med. Gazette, 1909, xliv, p. 241.

Idem. Studien über pathogene amoben. Arch. f. Schiff- u. Tropen-Hyg., 1909, Bd. xii, Beihest, 11, p. 18.

WILLIAMS, A. W., and GURLEY, C. R. Studies Research Lab., Dept. of Health, N. Y. City, 1908–09, vol. iv, p. 237.

WOOLEY, P. G., and MUSGRAVE, W. E. The Pathology of Intestinal Amœbiasis. Jour. Am. Med. Assoc., 1905, p. 1371.

ZANCAROL. Pathogenie des absces du foie. Rev. Centralbl. f. Bakt., etc., 1893, 1 Abt. xiv, p. 638.

ZAUBITZER, H. Studien ueber eine dom Strohinfus entaommene amœbe. Arch. f. Hyg., 1901, xlii, p. 311.

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